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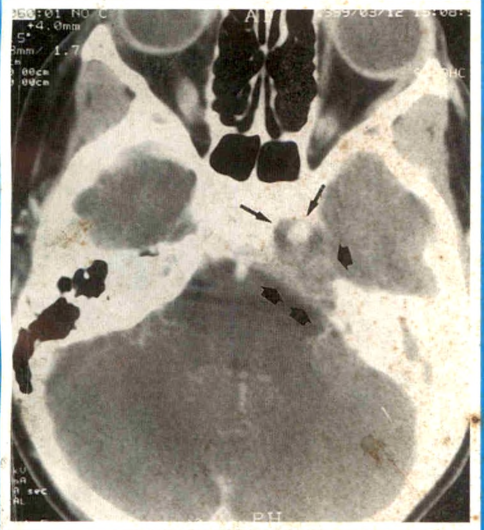

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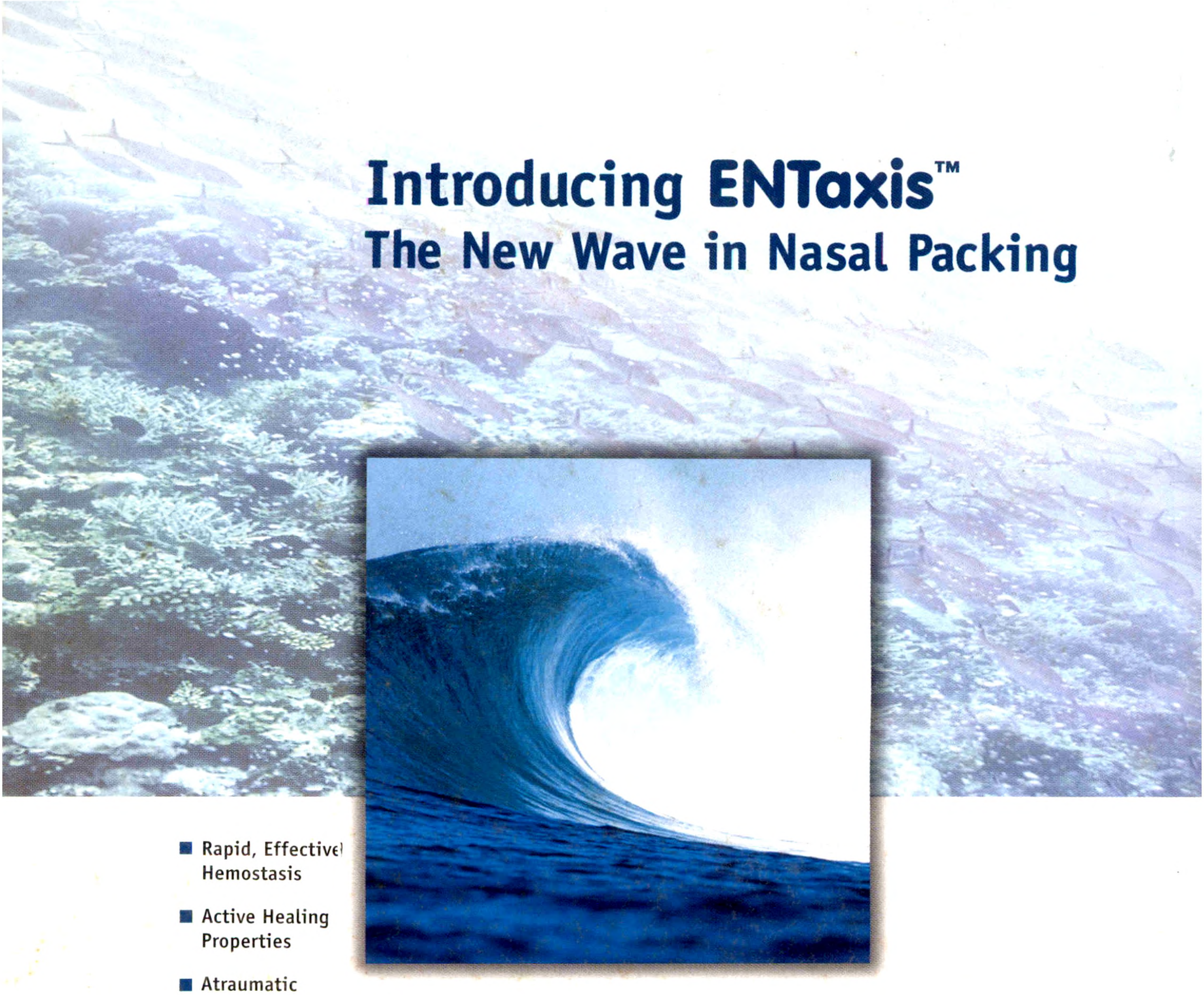

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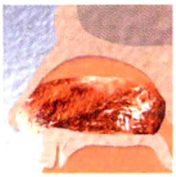

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PERSISTENT MEASLES VIRUS INFECTION AND OTOSCLEROSIS

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The cause of otosclerosis is still unknown. Recently, measles virus involvement has been implicated. The aim of this study was to analyze the presence of measles virus RNA within the otosclerotic focus and to evaluate the perilymphatic antibody pattern. Bone and perilymph specimens from 40 patients with the spontaneous form of otosclerosis and from control patients were investigated by reverse transcription polymerase chain reaction (RT-PCR), Western blot techniques, and cell culture. By the use of RT-PCR, measles virus RNA could be detected in 32 patients, but not in controls. Analysis of perilymph revealed the presence of antibodies to N, F_1, and M measles virus proteins in all cases, and antibodies against H protein in 2 additional cases. In preosteoblasts cultured from otosclerotic bone chips, no measles virus RNA could be amplified. We conclude that the spontaneous form of otosclerosis is, in the vast majority of cases, a measles virus–associated disease of the otic capsule.

KEY WORDS — measles virus, otosclerosis, perilymph, polymerase chain reaction, Western blot.

INTRODUCTION

Otosclerosis is a disease of the human temporal bone in which foci of bone resorption and reactive bone formation develop in the ontogenetically "weak" border region between bone and cartilage. In about 50% of cases, a familial incidence has been described, suggesting a role for hereditary and genetic factors.[1] Otosclerosis is more common in women, and pregnancy increases the progression of the otosclerotic lesion. It affects mainly whites and is often bilateral and symmetric. About 15 million people in the United States have otosclerosis, and it is considered to be among the most common causes of acquired deafness.[2] Ten percent of afflicted individuals experience conductive or mixed hearing loss because of gradual fixation of the stapes footplate by new bone formation. In the remaining cases, the lesion does not encroach on the oval window and is referred to as "histologic otosclerosis." In a small percentage of cases, otosclerosis can result in a progressive sensorineural hearing loss.

Morphological investigations have shown the presence of an inflammatory process within the temporal bone with distinct stages of bone resorption, new bone formation, and final eburnation of the affected bone. Metabolic disorders, vascular disturbances, mechanical stress, and toxic substances have been discussed as possible etiopathogenetic factors.[3] The inflammatory nature of this process has been clarified by immunohistochemical investigations that have revealed the presence of a variety of immunocompetent cells, including macrophages, HLA-DR–positive cells, and cells expressing beta-2 microglobulin, T-suppressor cells, or complement C3.[4,5] Deposits of immunoglobulins (IgG, IgM, and IgA) and complement C3 are present along the resorption lacunae, as well as in osteocytes and chondrocytes surrounding the destructive process.[5,6]

The origin of this chronic inflammatory process has still not been elucidated. The presence of autoantibodies to collagens II and IX in serologic specimens of otosclerosis patients has been reported.[7] An association of the Col IAI gene with a familial case of clinical otosclerosis has been reported.[8] In another bone remodeling disease, Paget's disease of bone, which shows morphological characteristics analogous to those of otosclerosis, paramyxoviral persistence has been described.[9,10] Investigations of otosclerotic tissue by electron microscopy have revealed in osteoblasts and preosteoblasts of otospongiotic foci the presence of filamentous structures morphologically similar to paramyxoviral nucleocapsid.[11] Immunohistochemical studies have confirmed the expression of measles virus nucleocapsid (N) and fusion (F) proteins in chondrocytes, in osteocytes, in osteoblasts, and in connective tissue, especially in otospongiotic (resorptive) areas.[12] Attempts to detect measles virus in otosclerotic bone by tissue culture and to localize the virus by in situ hybridization have failed up to now. By the use of reverse transcription polyme-

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From the Department of Otorhinolaryngology–Head and Neck Surgery, Technical University of Munich (Niedermeyer, Arnold, Schuster, Sedlmeier), and the Max-Planck-Institute of Biochemistry, Department of Virology, Martinsried/Munich (Baumann, Kramer, Neubert, Sedlmeier), Munich, Germany. This project was supported by grant H15-96 from the Kommission für Klinische Forschung, Germany.

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PRIMERS USED FOR MEASLES VIRUS RT-PCR AND SOUTHERN BLOT (MV 12)

MV 6: 5'-CTG AGC CTT GTT CTT CCG A-3'
MV 7: 5'-GCT CAG CTG ACC TTC GAC TG-3'
MV 8: 5'-GCA TCT GAA CTC GGT ATC AC-3'
MV 9: 5'-CTC CGA TGC AGT GTC AAT GT-3'
MV 10: 5'-AGA ATG AGC TAC CGC GAT TG-3'
MV 12 probe: 5'-AGA GCA AGT GAT GCG AGA GCT-3'

RT-PCR — reverse transcription polymerase chain reaction.

rase chain reaction (RT-PCR), measles virus RNA has been amplified from otosclerotic tissue, both from fresh frozen bones and from archival temporal bone specimens.[13,14]

Since the otosclerotic focus usually is in direct contact with the inner ear space, an immunologic reaction of the inner ear immune system, assumed to be localized within the endolymphatic sac, may be observed.[15] Localization of measles virus and measles virus protein expression within the otosclerotic focus may lead to an immunologic reaction within the inner ear immune system with a humoral and cellular response. In previous examinations, we have demonstrated that in patients with otosclerosis, the amount of specific measles virus IgG in comparison with total IgG is higher within the perilymph than within the serum.[16]

The aims of this study were 1) to analyze otosclerotic specimens for the presence of measles virus RNA and 2) to analyze perilymph for the presence of anti–measles virus antibodies.

PATIENTS, MATERIALS, AND METHODS

Included in the study, after informed consent, were 40 individuals (28 women and 12 men) with an average age of 42 years (range, 25 to 67 years) and a known clinical diagnosis of nonfamilial otosclerosis who were undergoing stapedectomy and, as controls, 2 patients with malignant tumors of the temporal bone who were undergoing labyrinthectomy, 4 patients who were undergoing cochlear implantation after temporal bone fracture, and 5 patients with Meniere's disease who were undergoing vestibulotomy. All patients and controls had a known history of measles virus infection. Serologic titers of anti–measles virus IgG and IgM, determined routinely for all individuals, revealed no evidence of acute measles virus infection, but in all individuals, measles virus IgG was detected in the serum.

Stapes footplates, crura, and bone chips from the mastoid bone from patients with otosclerosis and from patients with malignant tumors of the temporal bone, removed during stapedectomy and/or surgery of the labyrinthine block, respectively, were immediately snap-frozen and stored in liquid nitrogen. Measles virus (Edmondston strain, American Type Culture Collection)–infected and –uninfected Vero cells (American Type Culture Collection) were used as positive and negative controls.

Using only the capillary force for sampling, we sampled rising perilymph (1 to 2.5 µL), from all patients at the time of opening the oval window (stapedectomy for patients with otosclerosis and with malignant tumors of the temporal bone, vestibulotomy for patients with Meniere's disease, and opening of the basal turn of the cochlea for patients undergoing cochlear implantation). Glass capillary tubes were used (Clark Electromedical Instruments, Pangbourne, England). The glass capillary tubes were prepared by pulling them out over a gas flame so that they had an inner diameter of about 100 µm. Sampling was done at the footplate level, and capillary tubes were never introduced into the vestibulum. This procedure was approved by the ethics committee of the Technical University of Munich (December 20, 1999). The perilymph samples were stored at –20°C after gentle centrifugation.

Measles Virus RT-PCR. Total RNA was extracted by a previously published process.[13] Briefly, the footplates were pulverized and the total RNA of the footplates and the negative controls (crura and chips of the mastoid bone) was extracted with a modified guanidinium thiocyanate–phenol–chloroform method or with the High Pure RNA Extraction Kit (Boehringer, Mannheim, Germany) according to the manufacturer's recommendations.[17] Isolated RNA was reverse-transcribed with oligo-dT or random hexamer primers (RT-Kit, Boehringer). Measles virus–specific PCR primers were designed on the basis of published sequence information from the GenBank/EMBL data bank and synthesized on an oligonucleotide synthesizer (see Table). Standard PCR mix (50 µL total volume) contained 2 µL of complementary DNA (cDNA) from the RT reaction and measles virus–specific primers, and amplification was done with 35 cycles at 58°C annealing temperature in a TC1 thermocycler (Applied Biosystems, Freiburg, Germany). To increase sensitivity and specificity, we subjected 1 µL of the amplification product to 35 further amplification cycles with internal primers. The PCR products were separated on a 2% agarose gel, and Southern blot hybridization was performed with an internal ^{32}P-labeled oligonucleotide (see Table) to confirm the specificity of the amplification product. As a quality control for RNA extraction and RT-PCR, actin cDNA from all RNA samples was amplified in a single-step PCR under the same PCR conditions as described above.

Western Blot. The measles virus IgG pattern was

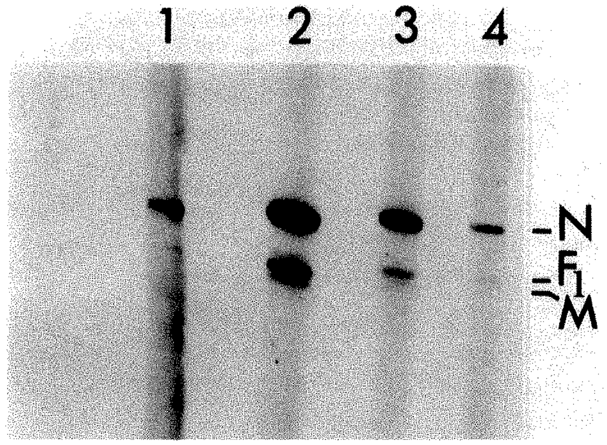

Fig 1. Mini-Western blot for detection of measles virus antibodies in patients and controls. Mini-Western blot from 12.5% polyacrylamide gel loaded with 640 ng of protein from measles virus–infected cells per lane. Strips with serum (lane 2) and perilymph (lane 1) samples from patient with otosclerosis and strips with serum (lane 3) and perilymph (lane 4) samples from patient with Meniere's disease. Original size of mini-Western blot strips is 3.2 × 35 mm.

analyzed by previously established mini-Western blots and midi-Western blots. Briefly, in the mini-Western blot system, proteins extracted from measles virus–infected Vero cells were separated by horizontal sodium dodecyl sulfate polyacrylamide gel electrophoresis on a 12.5% gel with the Pharmacia Phast system (Pharmacia, Freiburg, Germany). Upon electrotransfer of the proteins onto nitrocellulose (Immobilon P, Millipore, Eschborn, Germany), the blots were incubated with 0.5 µL of perilymph from patients with otosclerosis (n = 10), and negative controls from patients with malignant tumors of the temporal bone (n = 2) were incubated in 650 µL of probe buffer. As serum positive and negative controls, sera from patients with acute measles infection (n = 2) and sera from infants (n = 4) with no clinical or serologic evidence of a previous measles virus infection were used. As controls for the extracted measles virus proteins, monoclonal antibodies against measles virus proteins N, F, H, M, and P (supplied by F. Wild, Lyon, France; E. Norrby, Stockholm, Sweden; and V. ter Meulen, Würzburg, Germany) were used. Signal detection was done with the Enhanced Chemiluminescence Detection Kit (Amersham Life Sciences, Freiburg) according to the manufacturer's instructions.

To improve the resolution of the protein bands, we established a modified vertical midi-Western blot system. On a vertical 10% sodium dodecyl sulfate polyacrylamide gel, measles virus proteins were separated under exactly defined conditions, and the proteins were blotted onto nitrocellulose membrane (Immobilon P, Millipore) overnight by electrotransfer. Perilymph (1 µL) from all patients and from the negative controls was added to 1 mL of sample buffer, and the blots were incubated overnight at room temperature with gentle shaking. Signal detection was done as described above.

Cell Culture. Preosteoblasts obtained from otosclerotic bone chips and outer ear canal bone chips of the same patient (n = 5) were grown in Dulbecco's modified Eagle's medium (Sigma, Homburg, Germany) supplemented with 10% heat-inactivated fetal calf serum and 1% antibiotics (penicillin, streptomycin) and harvested after 3 to 8 weeks. RNA extraction and RT-PCR were done as described above.

RESULTS

RT-PCR. With the nested RT-PCR system, we were able to detect measles virus–specific sequences in the footplate samples from 32 of the 40 patients with otosclerosis. Southern blot hybridizations confirmed that the amplification products were specific for measles virus RNA. Control tissue from these patients and footplate material from controls consistently failed to yield any amplification products on RT-PCR. Actin cDNA, included as a control, was amplified in all cases.

Mini-Western Blot. In all 10 patients and in the positive controls, antibodies to measles virus proteins N, P, F1, and M could be detected in the serum. The serum-positive control showed a band pattern very similar to that of otosclerosis patients, but the intensity of the N protein band was slightly enhanced in the control group. Two of 4 serum-negative controls showed a very weak reaction with measles virus N protein, but no reaction with the other 5 measles virus proteins. The analysis of the perilymph samples revealed in all otosclerosis cases and in the negative controls the presence of 3 bands corresponding to measles virus N, F1, and M proteins. However, the band intensity of the negative controls was reduced in comparison with the bands obtained with the perilymph from patients with otosclerosis (Fig 1).

Midi-Western Blot. Midi-Western blot analysis confirmed the presence of antibodies against N, F, P, M, and, additionally, H proteins in all serum samples. Even in this midi-scale system, antibodies to measles virus proteins could be detected in the perilymph; in all otosclerosis patients and controls, antibodies to N, F1, and M proteins could be identified, while only in 2 cases were additional bands at the level of H present. L protein could not be detected in any perilymph. Concerning serum-positive and serum-negative controls, the results of the mini-Western blot were confirmed. The signal intensities obtained with perilymph were significantly weaker than those obtained with serum, but there was no remarkable dif-

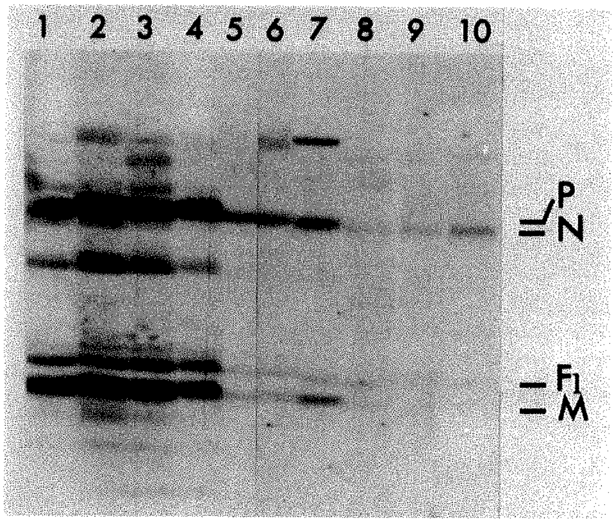

Fig 2. Anti–measles virus IgG pattern in serum and perilymph. Detection of antibodies to measles virus proteins N, P, F_1, and M in serum from patients with bone samples positive for measles virus by reverse transcription polymerase chain reaction (RT-PCR; lanes 1 through 4). Analysis of perilymph sample from measles virus RT-PCR–positive bone case revealed additional band over P (arrow) in lane 7. Perilymph samples from Meniere's patient (lane 9) and measles virus RT-PCR–negative otosclerosis patient (lane 10).

ference in the general signal pattern between perilymph and serum. Perilymph blots of patients and controls with measles virus–negative RT-PCR findings showed an antibody pattern similar to that of the measles virus RT-PCR–positive cases, but signal intensities were reduced in the measles virus RT-PCR–negative cases. In 2 otosclerosis cases, additional bands were observed, in contrast to the cases of Meniere's disease (Fig 2). Identification of the bands was more precise than with the mini-gel system.

As expected, perilymph sampling never led to any sensorineural hearing impairment in any patient, thanks to the great care taken and the small amount of perilymph removed (1 to 2.5 µL of rising perilymph from a total perilymph volume of 200 to 220 µL).[18,19]

Cell Culture. After 12 weeks, the cells showed spontaneous calcification. There was no morphological difference between the cell culture obtained from the otosclerotic focus and the culture obtained from the control tissue (outer ear canal). After 3 to 8 weeks, no measles virus cytopathic effect was visible. Furthermore, measles virus RNA could not be detected by RT-PCR, whereas the positive control (measles virus–infected Vero cells) was consistently positive. Actin cDNA was amplified in all 5 cases examined.

DISCUSSION

Our investigations provide evidence for a measles virus persistence within the temporal bone of patients with clinical otosclerosis. In 32 of 40 cases, measles virus RNA–related sequences were found. These results corroborate those of our previous investigations, in which we demonstrated in 13 of 14 cases the presence of measles virus RNA within otosclerotic bone chips.[16,20] Our data are in good agreement with a report of McKenna et al[14] in which measles virus RNA was detected by RT-PCR in archival temporal bone specimens showing an otospongiotic focus.

Detection of viral RNA denotes at first glance only the presence of measles virus within the examined bone specimens. However, extracellular measles virus would only be encountered immediately after an exposure to the virus, and the virus would be eliminated quickly by the immune system. A positive PCR reaction therefore indicates that a measles virus infection of the temporal bone, ie, virus adsorption and penetration of the host cell, has occurred. With 80% of the examined specimens being positive for measles virus RNA and with no serologic signs of acute infection, the data further indicate that the measles virus RNA is in a persistent state with a strong reduction of viral replication and viral gene expression so that elimination of the infected cells by the immune system is avoided.

In 8 cases of otosclerosis, no measles virus sequence could be detected in the specimens, and there are several explanations that might account for this result. The tissue was either never infected by measles virus, or the infected cells were lost in the final stages of otosclerotic eburnation. This latter explanation is supported by the well-known morphological pattern in which in the late stage of otosclerosis only a very small amount of osteoblasts, blood vessels, or connective tissue cells can be found.[21] Furthermore, we cannot rule out the possibility that some of our samples did not contain otosclerotic tissue. Our experimental settings were designed for efficient RNA extraction and high sensitivity of the RT-PCR amplification. Fixation and decalcification of the bone specimen, needed for a histologic examination, were avoided, in contrast to the experiments of McKenna et al,[14] who used embedded material. Decalcification and celloidin embedding, however, can seriously damage RNA, which might account for the few negative results in McKenna's examinations. Finally, the size of the sample is usually very small (about 1 mm^3), and it is known that in persistent infections, the level of viral replication and transcription is very low. The number of measles virus templates in the RNA preparations could therefore be under the detection limit of RT-PCR. However, taken together, our results confirm that in the vast majority of cases of clinical otosclerosis, measles virus RNA is present within the otosclerotic focus.

Various investigations in the past have demonstrated that an immunologic reaction within the inner ear may be induced by antigens. The site of humoral and cellular immunologic activity within the inner ear, including humoral and cellular response, has been shown to be the endolymphatic sac.[15,22] These observations have been confirmed by experiments of immunologic challenge using keyhole limpet hemocyanin in guinea pigs.[23] In contrast, scala tympani inoculation of cytomegalovirus following systemic infection in guinea pigs with cytomegalovirus has not led to antibody production either in the serum or in the perilymph. The authors have discussed that the immunologic reaction is delayed and needs more time to develop.[24] In humans, evidence of such an immune response of the inner ear has been found in patients affected by otosclerosis. In the perilymph of patients with otosclerosis, antibodies to measles virus antigens have been found, and the fact that the percentage of anti–measles virus IgG in total IgG was higher in the perilymph than in the serum indicates a local production of antibodies.[16]

In the actual investigation, we could determine the presence of IgG antibodies to the measles virus proteins N, P, F1, H, and M in the serum of all patients and controls. This is not surprising and is a result of vaccination or subclinical or clinical measles virus infection, which lead to lifelong immunity with constantly present IgG antibodies. With the mini-Western blot system, very small sample volumes can be analyzed, although the exact distinction and identification of the obtained signals is sometimes difficult because of the small gel dimensions (about 2.4 × 3.5 cm). When perilymph was analyzed in the mini-Western blot system, bands for N, F1, and M measles virus protein could be detected. The results are in good agreement with our previous finding that the relative amount of anti–measles virus IgG in total IgG is higher in the perilymph than in the serum.[16]

The midi-Western blot system confirmed the antibody patterns observed in the 10 cases that were analyzed by the mini-Western blot system. In all patients and controls, signals for N, P, F1, H, and M proteins were obtained with the serum samples. In contrast, when perilymph was investigated, only signals for N, F1, and M proteins were seen, and in 2 cases, reaction products at about H level were observed. The absence of H reaction product in the 40 perilymph samples might be due to the very small sample volume, which results in a weak signal intensity. Furthermore, measles virus transcription in infected cells is characterized by a 3'-5' transcription gradient with strong expression of the N and P genes and weaker expression of the following genes, which may explain the observed signal distribution.[25] Additionally, the lack of anti-M and anti-H IgG in the perilymph could be a consequence of reduced or missing H protein synthesis in persistently infected cells within the otosclerotic focus, as this protein is not necessary for viral persistence. Mutations within the viral H gene are well-known characteristics of measles virus persistence, and these mutations are considered basic events in the etiopathogenesis of subacute sclerosing panencephalitis.[26] Sequence analysis of the measles virus RNA could elucidate the role of mutations in the expression pattern in the perilymph.

It is important to note that in the otosclerotic patients with negative measles virus RT-PCR results and in the controls (patients with malignant tumors or Meniere's disease), there was no striking difference in the antibody pattern of the serum and perilymph samples as compared to patients with positive measles virus RT-PCR results, although the signal intensity of the perilymph samples was reduced. However, it is difficult to quantify antibody amounts exactly by Western blot analysis, especially when very low sample volumes have to be handled. Previous enzyme-linked immunosorbent assay results showed an increased amount of measles virus–specific IgG in total IgG from the perilymph as compared to the serum in otosclerosis.[16] But one must take into account that part of the measles virus IgG in the perilymph derives physiologically from the blood, and blood contamination during sampling can never be completely excluded. The origin of the additional bands in the 2 measles virus RT-PCR–negative otosclerosis cases in contrast with the Meniere's cases is not clear. Similar bands were observed in the measles virus RT-PCR–positive cases. As mentioned above, sequencing of the measles virus may contribute to elucidating this problem.

Cells cultured from otosclerotic bone chips and control bone fragments of the outer ear canal showed calcification and expression of osteocalcin and osteonectin, which are characteristics of bone cells. Measles virus–related sequences could not be detected in RNA from these cells by RT-PCR, perhaps because of the absence of measles virus–infected cells within the samples. However, it must be considered that persistently infected cells within a mixed cell population proliferate with severely reduced growth rates compared to uninfected cells, as has been shown for subacute sclerosing panencephalitis.[27] Upon prolonged cultivation, persistently infected cells can therefore easily be outgrown by uninfected cells; this competition might account for the negative PCR results in the cell culture experiments. Establishment of a persistently infected osteoblast cell line and characterization of measles virus propagation in this cell line could be a step toward a better understanding of the effect of measles virus–infected cells in otosclerosis.

Hereditary and genetic factors in the development of otosclerosis have been widely considered in the literature, with multiple incidences in certain families supporting this hypothesis.[1] A genetic predisposition, influenced by one or more unidentified factors, might lead to the development of the characteristic otospongiotic process in the border region between bone and cartilage of the otic capsule. Genetic factors may account for an enhanced affinity for infection of cells in particular regions, such as the globuli ossei, by certain viruses with specific tropism.[28] However, in our clinical practice, familial cases of otosclerosis are rare, and all patients described in this study had a spontaneous form of otosclerosis. One study localized an otosclerosis gene on chromosome 15q25-q26 by linkage analysis of a family with 16 members affected.[29] Other studies have shown the presence of antibodies to collagen types II and IX.[7,30] However, it is not clear whether antibodies are the consequence or the cause of the inflammatory process. If the latter, inflammatory reactions should also be observed in various other locations of the skeleton.

CONCLUSION

In conclusion, our results strongly support the hypothesis that measles virus is associated with spontaneous cases of otosclerosis. We found measles virus RNA in 80% of otosclerotic specimens, and antibodies to various measles virus proteins could be detected within the perilymph. However, measles virus RNA could not be found in cells cultured from the otosclerotic focus. The question still remains whether measles virus is a factor that can initiate proliferation and aggressive infiltration of connective tissue and blood vessels into the bone of the otic capsule, or whether otosclerosis is an epiphenomenon of measles virus. Identification and characterization of the infected cells within the otosclerotic focus, in combination with analysis of persistent measles virus infection in osteoblasts, will contribute to a better understanding of the role of measles virus in otosclerosis.

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COSMETIC FACIAL SURGERY

The Indian Society of Facial Plastic and Reconstructive Surgery Inaugural Conference and Workshop on Cosmetic Facial Surgery will be held December 7-9, 2001, in Indore, India. For information, please contact Dr Subramaniam Iyyer (subu@md3.vsnl.net.in) or Dr Brajendra Baser, 109 Trade House South Tukoganj Indore MP 452001, India (brajendrab11@hotmail.com, baser@vsnl.com).

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MECHANISM OF HEARING LOSS IN IRRADIATED EARS: A LONG-TERM LONGITUDINAL STUDY

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TAIPEI, TAIWAN

A 10-year longitudinal follow-up study of hearing was conducted in patients with nasopharyngeal carcinoma (NPC) in order to elucidate the mechanism of hearing loss in irradiated ears. Ten NPC patients were subjected to a battery of audiological tests before irradiation and 6 months, 5 years, and 10 years after irradiation. The tests included pure tone audiometry, tympanometry, eustachian tube function testing, and myringotomy to confirm middle ear effusion. The prevalences of otitis media with effusion (OME) were 25%, 25%, 40%, and 25% at the 4 testing periods described above, respectively. The prevalences of chronic otitis media were 0%, 0%, 15%, and 25%, respectively. In myringotomized ears (n = 17), the mean hearing levels for both air conduction and bone conduction were preserved from the preirradiation period to 10 years after irradiation. In contrast, in grommeted ears (n = 3), the mean hearing levels for both air conduction and bone conduction deteriorated progressively from the preirradiation period to 10 years after irradiation. We conclude that hearing can be preserved in NPC patients 10 years after irradiation if middle ear inflammation is well controlled. We do not recommend grommet insertion in irradiated NPC patients with OME, as it may result in persistent otorrhea and hearing deterioration.

KEY WORDS — grommet, hearing loss, irradiation, nasopharyngeal carcinoma, otitis media with effusion.

INTRODUCTION

Because of anatomic location, irradiation is by far the best treatment method for patients with nasopharyngeal carcinoma (NPC). To eradicate the tumor mass, the radiation field should cover a column from the base of the skull down to the clavicle level, including the skull base, blood vessels, nose and sinuses, salivary glands, pharynx, larynx, mandible, eustachian tube, and middle ear. The tumor mass itself may invade these areas, or irradiation may destroy them. Radiation is a known cause of otologic sequelae such as eustachian tube dysfunction, otitis media with effusion (OME), chronic otitis media (COM), and conductive or sensorineural hearing loss.[1-5] However, improved radiation techniques target the nasopharynx from 6 directions, so that the estimated dosage to the temporal bone is reduced to 70% of the total dosage, according to radiation isodose curve measurements. In terms of middle ear complications, we have already reported[4,5] that the causes of hearing loss in irradiated ears remain obscure because of the lack of a long-term longitudinal study. Four proposed causes have been as follows: 1) direct radiation injury to the inner ear or brain stem,[6] 2) obliterative endarteritis,[7] 3) eustachian tube dysfunction leading to COM,[2,4] and 4) ototoxicity caused by ear drops or cancer chemotherapy.[8]

The aim of this study was to elucidate the mechanism of hearing loss in irradiated ears of NPC patients by examining their audiograms over 10 years.

METHODS

Initially, 50 new NPC patients were subjected to a battery of audiological tests before undergoing irradiation. The tests included pure tone audiometry, tympanometry, eustachian tube function testing, and myringotomy to confirm middle ear effusion. Thirty-four patients completed their course of irradiation. Twenty patients survived for 5 years, and 14 survived for 10 years. However, only 10 patients had complete audiological test data from before irradiation and from 6 months, 5 years, and 10 years after irradiation, and these patients were enrolled in the study.[9,10]

RESULTS

Of the 10 patients, 7 were men and 3 were women. Their ages when NPC was diagnosed ranged from 22 to 62 years, with a mean of 42 years. The radiation dosages ranged from 70 to 80 Gy (mean, 76 Gy). The prevalences of OME were 25%, 25%, 40%, and 25% at the 4 testing periods of before irradiation and 6 months, 5 years, and 10 years after irradiation, respectively. The prevalences of COM were 0%, 0%, 15%, and 25% at the above 4 periods, respectively (Fig 1). Seventeen ears received myringotomy for either diagnosis or treatment, and 3 ears received grommet insertion.

The myringotomized ears maintained their mean

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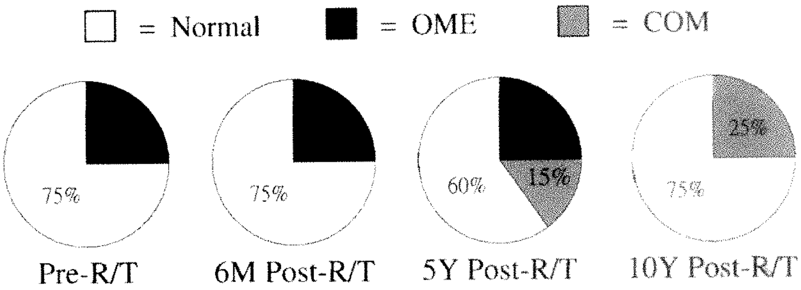

Fig 1. Prevalence of middle ear complications in 10 nasopharyngeal carcinoma patients (20 ears) before irradiation (R/T) and at 6 months, 5 years, and 10 years after irradiation. OME — otitis media with effusion; COM — chronic otitis media.

hearing level for air conduction for the frequency range from 250 to 4,000 Hz from before irradiation to 10 years after irradiation ($p > .05$). They also maintained their mean hearing level for bone conduction from before irradiation to 10 years after irradiation. At the 4 testing periods, for the myringotomized ears, the mean hearing thresholds at 5 frequencies (250, 500, 1,000, 2,000, and 4,000 Hz) were 30, 32, 35, and 38 dB hearing level (HL) for air conduction, and 17, 21, 22, and 24 dB HL for bone conduction, without a significant difference ($p > .05$) over the decade (Fig 2).

However, in the grommeted ears, the mean hearing levels for air conduction and bone conduction deteriorated progressively from before irradiation to 10 years after irradiation. The mean hearing thresholds for 5 frequencies of the grommeted ears at the above 4 periods were 23, 43, 55, and 66 dB HL for air conduction and 11, 26, 26, and 37 dB HL for bone conduction, respectively. These figures represent a significant hearing deterioration ($p < .05$) over the decade (Fig 3).

DISCUSSION

The temporal bone is relatively resistant to radiation insult unless the estimated dosage exceeds 65 Gy,[11] in which case radiation injury to the inner ear may occur. Of the 4 proposed mechanisms associated with postirradiation hearing loss mentioned above, the first is direct injury with early onset,[6] and the second is obliterative endarteritis with delayed onset.[8] Direct injury may be related to the radiation dosage, as exhibited in reirradiated NPC patients. At our hospital, the maximum dosage for NPC is maintained around 70 to 80 Gy, providing a dosage of 49 to 56 Gy to the inner ear and brain stem, which is not enough to provoke radiation injury. The second mechanism of hearing loss, obliterative endarteritis, was indicated in a previous study to cause a delayed (12 years) and sudden-onset sensorineural hearing loss after irradiation.[5]

This study suggests, surprisingly, that hearing can be preserved in myringotomized ears of long-term survivors of NPC (Fig 2). This finding indicates that neither direct radiation injury nor obliterative endarteritis is the main cause of hearing loss in this sample of long-term survivors of NPC. Instead, eustachian tube dysfunction leading to COM, with superimposed ear drop ototoxicity (if ear drops have been used), could be the cause. In a long-term study of eustachian tube function in NPC patients, tubal dysfunction consisting of organic obstruction and functional impairment was improved 10 years after irradiation,[4] resulting in the subsidence of otitis media and the preservation of hearing. Therefore, preservation of tubal function in irradiated survivors of NPC may

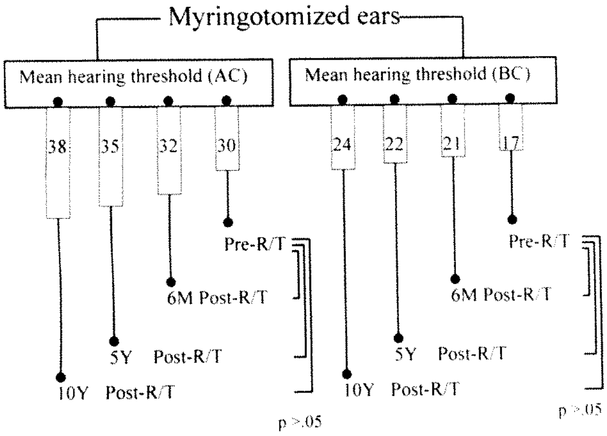

Fig 2. Mean hearing thresholds (numbers in column, decibels hearing level) for air conduction (AC) and bone conduction (BC) in myringotomized ears (n = 17) show how hearing was preserved from before irradiation to 10 years after irradiation.

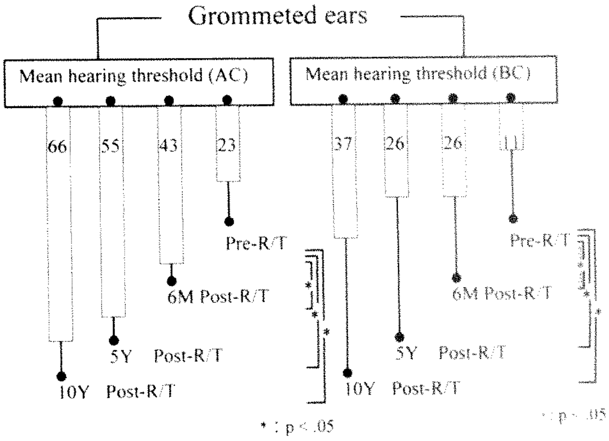

Fig 3. Mean hearing thresholds (numbers in column, decibels hearing level) for air and bone conduction in grommeted ears (n = 3) show how hearing deteriorated from before irradiation to 10 years after irradiation.

prevent the development of COM and avoid the subsequent use of ear drops. This study recommends that 1) the maximum radiation dosage be 70 Gy and 2) repeated myringotomies replace grommet insertion in NPC patients with OME, as grommets may induce otitis media from nasopharyngitis by opening the eustachian tube.[3]

In 10-year survivors of NPC with a normal appearance of the eardrum, a hearing threshold the same as the preirradiation threshold is possible. This finding is quite encouraging and shows that like tubal dysfunction, hearing loss in irradiated NPC patients is transient and reversible if otitis media is well controlled. In other words, the mechanism of hearing loss in irradiated ears mainly arises from middle ear complications, possibly aggravated by grommet insertion, that result in intractable otitis media, labyrinthitis, ear drop ototoxicity, and subsequent hearing impairment.

CONCLUSION

Hearing can be preserved in NPC patients 10 years after irradiation if middle ear inflammation is resolved. Accordingly, grommet insertion is not recommended in irradiated NPC patients with OME, as it may result in persistent otorrhea and hearing deterioration.

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SHORT- AND LONG-TERM RESULTS OF STAPEDOTOMY AND STAPEDECTOMY WITH A TEFLON-WIRE PISTON PROSTHESIS

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We analyzed the results of 604 cases of primary stapes surgery performed between 1974 and 1997 with replacement of the stapes by a 0.6- or 0.8-mm Schuknecht Teflon-wire piston. At long-term follow-up (1 to 21 years; mean, 7 years), the residual air-bone gap was 10 dB or less in 79% of the cases. The hearing results and postoperative complications were comparable to those reported by authors who used the same evaluation criteria. Although the aim of the surgery was to perform a small stapedotomy with a narrow footplate perforation (0.8 mm), a large stapedotomy or a stapedectomy was performed in 134 cases (22.2%) because of surgical or anatomic conditions. Our results show that the larger footplate perforations allowed a better correction of the air-bone gap at the lower frequencies. The ears with larger perforations did not show a higher incidence of sensorineural hearing loss.

KEY WORDS — stapedectomy, stapedotomy, teaching.

INTRODUCTION

The surgical technique for the correction of hearing loss caused by otosclerosis has quickly evolved from total extraction of the footplate, the so-called stapedectomy,[1] to a small perforation in the center of the footplate, the stapedotomy.[2] Today, some surgeons use small-diameter pistons inserted into the inner ear through a fenestra not larger than 0.4 mm.[3] Unless a laser is used, the footplate perforation and placement of the prosthesis demand a high level of technical skill. Several reports of stapedectomy results have been published, demonstrating the advantages of stapedotomy compared to other techniques.[2-4] However, series that include operations performed by residents and fellows in training are rare.[5,6]

The objective of this study was to evaluate the short- and long-term results of all cases of stapes surgery performed in our department between 1974 and 1997 and to address the question of whether stapes surgery can be taught safely. In every case, the aim of surgery was to perform a small stapedotomy, but because of surgical or anatomic conditions, a larger stapedotomy or a stapedectomy was necessary in about 1 out of 5 cases. These cases were initially considered as surgical accidents, but to our surprise, analysis showed that the functional results and the rate of complications were similar to those of small stapedotomies.

MATERIALS AND METHODS

We analyzed the results of 604 consecutive cases of otosclerosis that were treated surgically between 1974 and 1997 by 19 different surgeons, including residents in training. The series included 253 men (42%) and 351 women (58%). The age of the population at the time of surgery ranged from 17 to 72 years, with an average of 43.0 years (SD, 10.7 years). The diagnosis of otosclerosis was based on a clinical history of progressive hearing loss, normal otoscopic findings, a negative Rinne test, an audiogram showing a conductive hearing loss of >20 dB in the range of 0.5 to 2 kHz, and the absence of a stapedial reflex.

Surgical Techniques. All operations were performed as described by Schuknecht and Bentkover.[7,8] A transcanal approach through an ear speculum was standard. A posterior tympanomeatal flap was elevated, the stapedius tendon cut, and the crural arch fractured and removed. The footplate was perforated with a hand drill or a motor-driven, sharp cutting bur. The aim was always to perform a narrow perforation (0.8 mm). Accidentally, in cases of fracture or a floating footplate, a larger perforation or a stapedectomy was performed. First, cases were divided into 3 groups according to the size of the footplate perforation. We considered 1) small stapedotomies those with a 0.8-mm perforation, 2) large stapedotomies those with a perforation larger than 0.8 mm, and 3) stapedectomies those with total footplate removal. All footplates were reconstructed with a Schuknecht Teflon-wire piston. From 1974 to 1983, the wire was made of stainless steel; after 1983, it was made of platinum. A 0.6-mm-diameter piston was used in most cases, but a 0.8-mm piston was used in some cases of stapedectomy.

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TABLE 1. OVERALL RESULTS OF PRIMARY SURGERY AND RESULTS ACCORDING TO SIZE OF FOOTPLATE PERFORATION

	Overall	*Small Stapedotomies*	*Large Stapedotomies*	*Stapedectomies*
Short-term follow-up	n = 604	n = 470	n = 83	n = 51
rABG ≤10 dB	477 (79.0%)	361 (76.8%)	73 (88.0%)	43 (84.3%)
rABG >10 and ≤20 dB	96 (15.9%)	86 (18.3%)	6 (7.2%)	4 (7.8%)
rABG >20 dB	23 (3.8%)	19 (4.0%)	3 (3.6%)	1 (2.0%)
SNHL >60 dB	8 (1.3%)	4 (0.9%)	1 (1.2%)	3 (5.9%)
SNHL 4 kHz >20 dB	25 (4.1%)	11 (2.3%)	6 (7.2%)	8 (15.7%)
Cases requiring revision	32 (5.3%)	27 (5.7%)	3 (3.6%)	2 (3.9%)
rABG >20 dB	19	16	2	1
Vertigo	13	11	1	1
Long-term follow-up	n = 441	n = 342	n = 58	n = 41
rABG ≤10 dB	350 (79.4%)	271 (79.2%)	48 (82.8%)	29 (70.7%)
rABG >10 and ≤20 dB	55 (12.5%)	43 (12.6%)	5 (8.6%)	9 (22.0%)
rABG >20 dB	36 (8.2%)	28 (8.2%)	5 (8.6%)	3 (7.3%)
Cases requiring revision	39 (8.8%)	30 (8.8%)	6 (10.3%)	3 (7.3%)
rABG >20 dB	36	28	5	3
Vertigo	3	2	1	0

Number of cases with residual air-bone gap (rABG) of ≤10 dB is not statistically different between groups, nor is number of cases with high-frequency sensorineural hearing loss (SNHL) of >40 dB.

In cases of large stapedotomy or stapedectomy, the oval window was sealed with a piece of earlobe fat tissue. The tympanomeatal flap was replaced, and the external ear was packed with silk strips and cotton soaked in neomycin sulfate, polymixin B sulfate, and hydrocortisone (Corticosporin). The packing was removed 5 to 7 days after surgery. In nearly all cases, surgery was performed under local anesthesia; in a few cases, upon the request of the patient, it was performed under general anesthesia. The procedure was reduced from one with a hospital stay of 5 days in 1974 to a mostly outpatient procedure from 1994 onward.

Evaluation of Results. Preoperative audiograms (Maico audiometer) were performed in a soundproof room the day before surgery. A short-term evaluation, including a postoperative audiogram, was performed 6 weeks after surgery. As a rule, our patients have subsequent yearly audiograms. The long-term results given here are based on the latest available audiogram, obtained 1 to 21 years (mean, 7 years) after surgery. Two values were considered: 1) the 3-frequency average air-bone gap (0.5, 1, and 2 kHz), based on the postoperative air and bone conduction thresholds, and 2) the postoperative bone conduction threshold at 4 kHz.

Analysis. The Newman and Keuls test was used to determine significant differences between variables involving frequency data, and an analysis of variance was used for quantitative data. The assumption of equal variances was tested with an F test.[9] A probability value (p) of less than .05 was the level of significance used for all hypothesis testing.

RESULTS

It was possible to perform a small stapedotomy in 470 cases (77.8%). A large stapedotomy was performed in 83 cases (13.7%), and a stapedectomy in 51 (8.4%).

Short-term Evaluation. The 604 patients were seen 6 weeks after surgery. The short-term results are shown in Table 1. The number of cases with a residual air-bone gap (rABG) of ≤10 dB did not show a statistically significant difference between the small stapedotomies (76.8%), large stapedotomies (87.9%), and stapedectomies (84.0%), but the mean (±SD) residual air-bone gap was statistically larger for small stapedotomies (6.2 ± 6.3 dB) than for large stapedotomies (3.5 ± 4.4 dB) or stapedectomies (3.3 ± 4.6 dB; $p < .01$). In the high-frequency range (4 kHz), the gain was significantly larger in the group of small stapedotomies (20.1 ± 12.3 dB) than in the other two groups (large stapedotomies, 15.2 ± 12.1 dB; stapedectomies, 15.6 ± 12.3 dB; $p < .01$).

The mean postoperative lowering of the bone conduction threshold at Carhart's notch was 3.0 ± 9.1 dB.

A high-frequency sensorineural hearing loss (SNHL) of >20 dB occurred in 25 cases (4.1%), and a low- and high-frequency (total) SNHL of >60 dB occurred in 8 (1.3%). The occurrence of SNHL was slightly more frequent and the loss (mean, 15.7 ± 12.8 dB) was greater in the groups of stapedectomies

TABLE 2. INDICATIONS FOR EARLY AND LATE REVISION SURGERY AND OPERATIVE FINDINGS ACCORDING TO SIZE OF PERFORATION

		Small Stapedotomies		*Large Stapedotomies*		*Stapedectomies*	
Indications for Revision	*Operative Findings*	*Early*	*Late*	*Early*	*Late*	*Early*	*Late*
Conductive hearing loss (n = 55)	Fibrous tissue	11	13	1	3	1	1
	Incus erosion	0	9	0	2	0	1
	Loosened loop	4	1	0	0	0	0
	Displaced prosthesis	0	2	0	1	0	1
	Reobliteration	0	2				
	Fixed malleus	2	0				
Vertigo (n = 16)	Excessively deep prosthesis	8	0				
	Granuloma	5	0	0	1	0	1
	Fibrous tissue			0	1		

Most cases of persistent or delayed recurrent conductive hearing loss resulted from excessive fibrous tissue and incus erosion, respectively. Vertigo was mainly due to retraction of incus by fibrous tissue pulling piston deeply into vestibule.

and large stapedotomies than in the group of small stapedotomies, but the differences did not reach the level of significance.

An early revision surgery was scheduled in 32 patients up to 6 weeks after surgery, including 19 of the 23 patients with an rABG of >20 dB and 13 with persistent postoperative vertigo. The results of revision surgery are reported below.

Long-term Evaluation. Data from 441 cases (73%) evaluated 1 to 23 years (mean, 7 years) after surgery are available. As there is no statistical evidence that the hearing results vary according to the time between surgery and the long-term follow-up, the analysis was made on the sample as a whole, and the sample was not broken down into subgroups according to the different lengths of time (correlation $R^2 = .106$; $p < .05$).

The rABG was ≤10 dB in 350 cases (79.4%; Table 1). The mean rABG was slightly larger for small stapedotomies (5.2 ± 7.8 dB) than for large stapedotomies (2.9 ± 5.9 dB) and stapedectomies (3.8 ± 6.7 dB), but these differences are not statistically significant.

A late revision surgery was scheduled in 39 cases, 1 to 23 years after the initial surgery (in 36 cases because of a persistent hearing loss, and in 3 because of progressive vertigo).

TABLE 3. SHORT- AND LONG-TERM RESULTS OF REVISION SURGERY

	Short-term (n = 71)	*Long-term (n = 53)*
rABG ≤10 dB	41 (57.7%)	37 (69.8%)
rABG >10 and ≤20 dB	18 (25.4%)	8 (15.1%)
rABG >20 dB	11	8
SNHL >60 dB	1 (1.4%)	
SNHL 4 kHz >20 dB	9 (12.7%)	

Number of cases with rABG of ≤10 dB is significantly lower than that after primary surgery.

Based on the first postoperative audiogram and the last available audiogram in each of these 441 cases, a mean annual hearing loss was calculated. The loss was 0.10 ± 3.59 dB per year in the low-frequency range (0.5, 1, and 2 kHz) and 0.6 ± 4.9 dB per year in the high-frequency range (4 kHz).

Revision Surgery. In total, 71 of the 604 cases (11.8%) required revision surgery: 57 of the 470 small stapedotomies (12.1%), 9 of the 83 large stapedotomies (10.8%), and 5 of the 51 stapedectomies (9.8%). A sole revision was necessary in 60 cases, 2 revisions in 9 cases, and 3 in 2 cases. Of the total of 71 revisions, 32 were early revisions, performed up to 6 weeks after the primary surgery, and the other 39 were late revisions, performed from 1 to 23 years after the primary surgery. Indications for early revision included persistent hearing loss in 19 cases and persistent postoperative vertigo in 13. Indications for late revision included recurrent hearing loss in 36 cases and late progressive vertigo in 3. Most cases of persistent hearing loss were due to fibrous tissue fixating the prosthesis. Most cases of progressive hearing loss were due to fibrous tissue fixating the prosthesis or to erosion of the long process of the incus. Persistent postoperative or late progressive vertigo were most frequently due to a prosthesis deeply settled into the oval window, sometimes secondary to retraction of the long process of the incus by fibrous tissue (Table 2). The revision consisted of dissection of the fibrous tissue and repositioning of the prosthesis. In 7 cases, the incus was eroded or fixed, and therefore, the piston was attached to the malleus. A Teflon disc was placed around the piston to prevent further development of fibrous tissue. The size of the oval window fenestra was not modified.

The results are shown in Table 3. The 71 cases were seen 6 weeks after the last (if multiple revisions were performed) revision surgery. An rABG of ≤10 dB was obtained in 41 cases (57.7%). The mean rABG (10.8 ± 9.7 dB) was significantly larger, and the gain in the high-frequency range (14.7 ± 11.9 dB) lower, than those observed after primary surgery ($p < .01$).

A high-frequency SNHL of >20 dB occurred in 9 cases (12.7%). This rate was not significantly different from that observed after primary surgery, but the hearing loss expressed in decibels was significantly greater. A low- and high-frequency SNHL of >60 dB occurred in 1 case, after a second revision.

Data from 53 cases were available for the long-term evaluation. The functional results were stable (Table 3).

DISCUSSION

A hearing gain with an rABG of ≤10 dB was achieved in 78.9% of the cases. The rate of success is somewhat lower than that reported by authors who calculate the postoperative rABG by subtracting the preoperative bone conduction threshold from the postoperative air conduction threshold.[4,6,10-12] This method tends to artificially improve the result and even to produce overclosure of the postoperative rABG, since the bone threshold improves after surgery.[13] In our series, the mean bone threshold was as much as 3 dB lower than the preoperative threshold. In other series, the improvement in bone conduction is even greater.[14] Therefore, our results should be compared to and are similar to those reported by authors who use the same criteria of evaluation.[15] The rate of complications such as immediate postoperative SNHL, vertigo, and persisting or delayed-onset conductive hearing loss is also comparable to those of many other recent reports.[12,16-18]

A yearly visit was recommended to all of our patients. Long-term evaluation data (1 to 21 years after surgery; mean, 7 years) were available in 441 cases. The percentage of cases with an rABG of ≤10 dB was similar to that observed in the early postoperative period. Therefore, the functional results are stable over time, whatever the size of the oval window fenestration. On the basis of the bone thresholds of the first postoperative audiogram and the last available audiogram, it was also possible to calculate the annual hearing loss of the operated ears. Our findings confirmed previous reports[16,18] showing that the annual hearing deficit was equivalent in patients operated on for otosclerosis to that observed by Osterhammel and Osterhammel[19] in a normal population 45 to 65 years old: 0.4 dB in the low- and 0.8 dB in the high-frequency range. Therefore, it seems that neither otosclerosis nor the piston inserted into the inner ear accelerates the physiological hearing loss due to aging.

Since Schuknecht and Applebaum[2] reported, in 1969, their excellent results achieved by introducing a small-diameter Teflon-wire piston through a small fenestra in the footplate, the so-called "stapedotomy" has become the best corrective procedure for stapes fixation caused by otosclerosis. According to Schuknecht,[8] this technique reduces many of the complications of stapedectomy, such as postoperative vertigo, reparative granuloma, and fibrous fixation of the lenticular process to the promontory. In our series, the aim of the surgery was always to create a small (0.8 mm) footplate perforation. But in about 1 of 5 cases, a larger stapedotomy or a stapedectomy was performed because of a floating or fractured footplate. The incidence of larger-than-desirable fenestrae reported by authors using a $CO2$ or argon laser to perforate the footplate is lower than that observed in our series.[20] Since, for economic reasons, most otologists will not have access to a laser once their residency has been completed, we deliberately chose not to use a laser for the training. To our surprise, the functional results of large stapedotomies or stapedectomies were not obviously worse than those achieved with small stapedotomies, and in contrast with previous studies,[8] the rate of complications was not significantly higher! In the low-frequency range, the hearing gain with stapedectomies and large stapedotomies was better than that achieved with small stapedotomies, while, as in other series,[10,21] it was somewhat lower in the high-frequency range. We believe that performing a small-fenestra stapedotomy requires a great deal of surgical skill to precisely perforate the footplate and to correctly position the prosthesis to avoid any parallax that could hinder the movement of the prosthesis. This part of the procedure is not easy for otologists in training. However, stapedotomy can be relatively safely taught to inexperienced surgeons, since in case of any surgical or anatomic difficulties, the supervising senior otologist can perform a partial or total removal of the footplate and still obtain good functional results without major complications.

As in other series,[10,15,21] the best low-frequency hearing gain was achieved in the group of stapedectomies. This finding could be explained by the experimental model of the middle ear developed by Rosowski and Merchant.[22] They demonstrated that the gain increases proportionally to the area of the movable surface in the oval window. In our series, the pistons used for the reconstructions had in nearly all cases the same diameter (0.6 mm). However, it is possible that the flexible fat tissue placed around the piston to seal a large oval window fenestra plays a role in the transmission of the sound to the inner ear by linking to the prosthesis and contributing to an increase in the vibrating surface area.

Revision surgery was necessary in 11.7% of cases, mostly because of persistent or late-onset recurrent conductive hearing loss. Some patients underwent 2 or 3 operations to finally obtain a good hearing gain that remained stable over time. The operative findings, such as prosthesis displacement or fixation by fibrous tissue or incus erosion, were similar to those described by many other authors.[23-26] After revision, an rABG of ≤ 10 dB was achieved in 58% and 70% of cases at the short- and long-term follow-ups, respectively. These results are better than those generally reported, which range from 39%[23] to 61%.[24] Most authors are hesitant to reoperate after a first revision procedure. Our series shows that a second or even a third operation is possible if hearing is not restored after a first attempt, and that although the results are not as good as those of primary surgery, they are still encouraging.

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TOTAL IMPLANTATION OF THE ACTIVE HEARING IMPLANT TICA FOR MIDDLE EAR DISEASE: A TEMPORAL BONE STUDY

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A subpopulation of hearing-impaired patients has conductive hearing loss that cannot be improved by classic tympanoplasty. Other patients have a mixed hearing loss and cannot be helped by present forms of ear surgery or by hearing aids. Possible help for some patients may come from current implantable hearing devices if these are modified for the patient's specific anatomic situation. The TICA LZ 3001 is a hearing implant for total implantation used to treat moderate to severe sensorineural hearing loss. Most patients who use it have a normal ossicular chain that allows coupling of the implant to the incus. The present temporal bone study demonstrates that the TICA can also be used in patients with an interrupted ossicular chain. If the incus long process shows a defect, the TICA may be coupled to the incus body, and connection between the stapes and the long process of the incus can be achieved with a commercially available titanium-angle prosthesis or liquid ionomeric cement. In cases of an absent incus, the coupling axis of the transducer may be coupled to the stapes head via a modified coupling element. With an absent stapes, the coupling axis may be coupled directly to the perilymph by a coupling element similar to a gold stapes prosthesis.

KEY WORDS — conductive hearing loss, implantable hearing aid, implantable hearing device, mixed hearing loss, TICA, totally integrated cochlear amplifier.

INTRODUCTION

A subpopulation of hearing-impaired patients has conductive hearing loss that cannot be improved by classic tympanoplasty. Other patients have a mixed hearing loss that cannot be helped by present forms of ear surgery or by hearing aids. Possible help for some of them could come from current implantable hearing aids if these are modified for the individual patient's anatomic situation.

Since 1984, pioneering work has been performed by Suzuki and Yanagihara, who have succeeded in implanting a partially implantable middle ear implant (P-MEI) in 70 patients with middle ear disease.[1] Their concept for an active hearing implant is based on a piezoelectric transducer with direct coupling to the stapes. The P-MEI consists of an implantable transducer and external, nonimplantable components (microphone, energy supply, and amplifier). The P-MEI is approved in Japan.

In 1988, at the University of Tuebingen, Germany, Leysieffer and Zenner initiated a program resulting in the development of the totally implantable hearing system Implex TICA LZ 3001[2-4] for sensorineural hearing loss (SNHL). Since 1998, the implant has been used in patients with moderate-to-severe SNHL.[5-7] In these cases, patients possessed a normal ossicular chain that allowed for coupling of the implant to the incus. The present temporal bone study shows that there is also a potential use of the TICA in selected patients with an interrupted ossicular chain. If the long process of the incus shows a defect, the TICA may be coupled to the incus body. Connection between the stapes and the long process of the incus can be achieved with a commercially available titanium-angle prosthesis or liquid ionomeric cement. At present, the implant and ionomeric cement are used in Europe. They are not yet approved in the United States by the Food and Drug Administration (FDA). However, it is hoped that the implant will receive FDA approval in the future. The TICA recently received IDE (investigational device exception) approval for clinical study in the United States.

In cases of a completely missing incus that are amenable to ossiculoplasty, the coupling axis of the transducer can be joined via a modified coupling element to the stapes head. In the case of a missing stapes superstructure, the coupling axis can be joined directly to the perilymph by use of a coupling element similar to a stapes prosthesis. This study systemati-

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From the Department of Otolaryngology, University of Tuebingen, Tuebingen (Maassen, Baumann, Zenner), and the Technical University of Esslingen, Esslingen (Lehner), Germany. H. Leysieffer is one of the chairmen of the Implex Company and a shareholder. H. P. Zenner is a shareholder in the Implex Company. M. M. Maassen, R. Lehner, and I. Baumann do not have a commercial relationship to the Implex Company.

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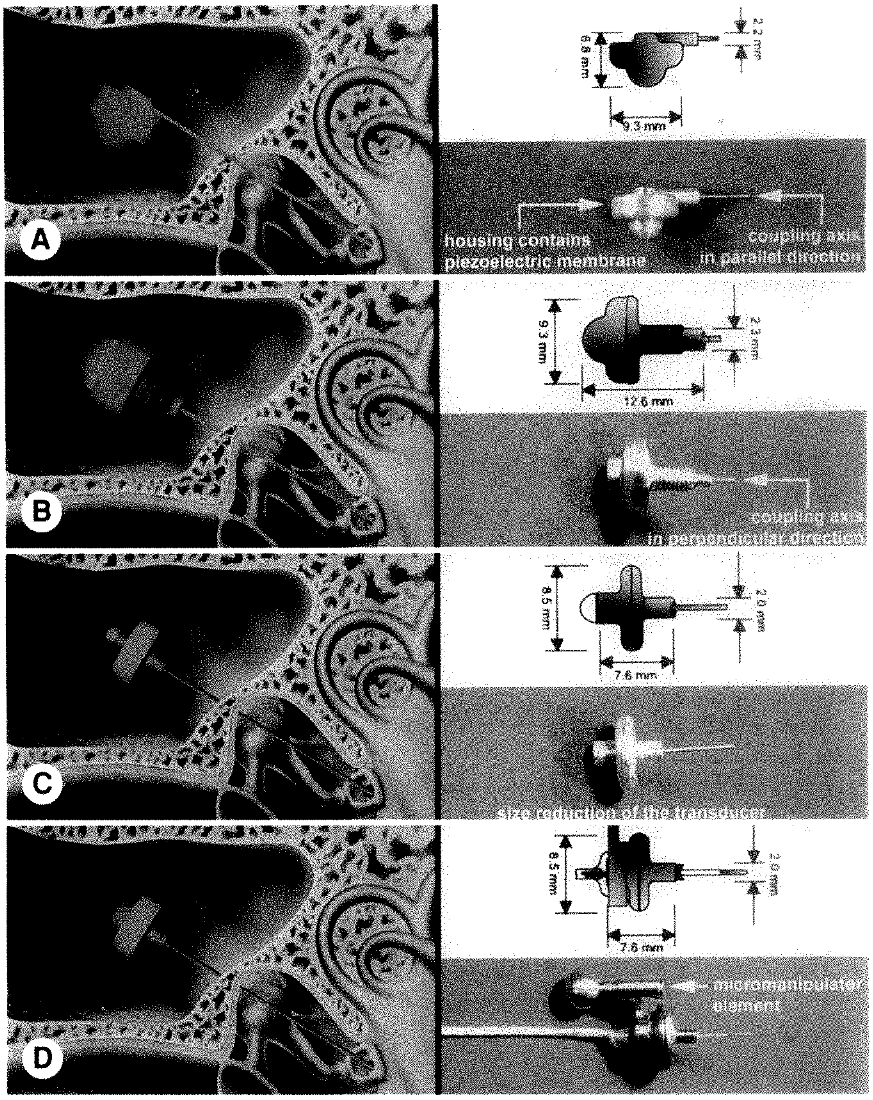
A
6.8 mm
2.2 mm
9.3 mm
housing contains piezoelectric membrane
coupling axis in parallel direction
B
9.3 mm
2.3 mm
12.6 mm
coupling axis in perpendicular direction
C
8.5 mm
2.0 mm
7.6 mm
size reduction of the transducer
D
8.5 mm
2.0 mm
7.6 mm
micromanipulator element

Fig 1. Transducer prototypes 1.1 to 4.0. Left side shows placement of transducer prototype into mastoid cavity. Right side shows construction diagram and photograph. **A)** Prototype 1.1. **B)** Prototype 2.3. **C)** Prototype 3.0. **D)** Prototype 4.0. Note that location of coupling axis in B-D allows direct coupling to perilymph (shown), and also indirect coupling to ossicular chain. Details are displayed in Fig 2.

cally evaluates the strategies for coupling the TICA to an interrupted ossicular chain. Furthermore, we present temporal bone data on a transducer shape that allows for implantation in most ears.

MATERIALS AND METHODS

Transducer prototypes were purchased from Implex Hearing Technology, Munich, Germany. Coupling elements were produced as previously described.[8] The aim of the present study was to assess new coupling sites for the electromechanical transducer. This could only be performed by taking the shape of the transducer into consideration. The transducer had to be shaped in such a way that it could be implanted in as many cases as possible. Therefore, different aluminum prototypes were produced. Before insertion, complete mastoidectomy was performed in 50 human temporal bones. In the posterior canal wall, an opening was created for insertion of the implantable microphone.[9] Measurements of anatomic distances were performed with micromeasurement tools (Kurz, Dusslingen, Germany). Anatomic distances from the microphone to the medial and lateral limits of the posterior canal wall, and from the microphone to the facial nerve, sigmoid sinus, aditus ad antrum, and ossicular chain were documented. From each implantation setting, a photograph was taken and videodocumentation (Sony VP-7040, Sony SV0-5800P) was performed.

All probabilities of success were calculated with 95% confidence intervals, which originated from Pearson-Clopper values. The true rate of the probability of success (ie, of in vivo implantations) is 95% in the region between the lower confidence limit (CLL) and the upper confidence limit (CLU). This means that the number of temporal bone specimens (n = 50) examined allowed a statistical estimation of all sizes of temporal bones, including in vivo temporal bones. The statistical calculations were generously performed by R. Lüdtke, MA, Institute for Medical Information Processing, University of Tuebingen, Germany.

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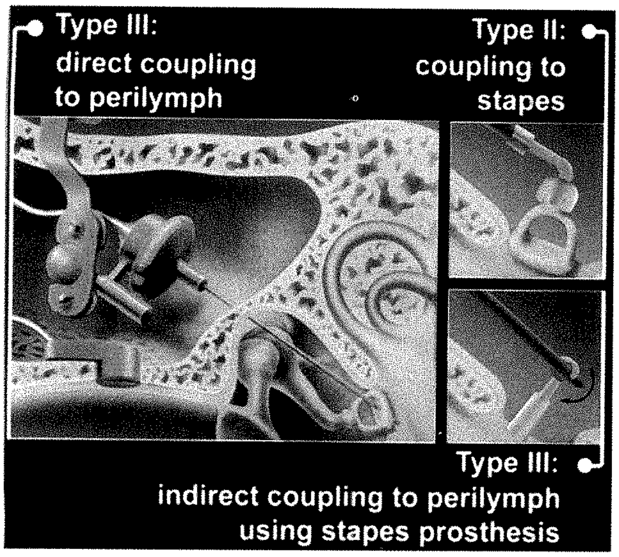

Fig 2. Coupling options of final transducer version. On left is direct coupling to perilymph of final version of transducer coimplanted with microphone. Coupling axis is inserted through perforation in stapes footplate into perilymph (type III). Surgically, this approach is difficult. At upper right is coupling to stapes with bell-shaped coupling element (type II). Lower right shows how, in order to overcome unwanted angle between coupling rod and oval window, stapes prosthesis can be crimped to coupling rod (type III).

RESULTS

Two basic coupling principles were examined: 1) direct coupling of the coupling axis to the perilymph, with or without a piston prosthesis as a coupling element, and 2) indirect coupling of the vibratory signal to the perilymph, with the ossicular chain or existing parts of it used as a linking element. Coupling to the incus body is an established surgical option for the TICA implant and can be achieved after mastoidectomy is performed. In the temporal bone specimen, the facial nerve was exposed. The distance between the facial nerve and the posterior tympanotomy was measured. The posterior tympanotomy opening showed a distance of 3.4 ± 1.2 mm (n = 17) to the facial nerve. In no case was the distance to the facial nerve smaller than 2 mm.

Localization and Orientation of Transducer. The transducer is placed into the mastoid cavity. In contrast to the published procedure,[10] the newly investigated coupling to the stapes head and perilymph could result in a different orientation of the transducer. Furthermore, the shape and length of the coupling axis had to be determined for the new transducer orientations.

To reevaluate the transducer's shape, we started with transducer 1.1 (Fig 1A). The axis was oriented parallel to the housing of the vibrating element, allowing the otosurgeon a good view through the posterior tympanotomy. Acoustic investigation, however, by laser Doppler velocimetry revealed that adequate signal transfer could not be achieved with a parallel coupling axis. Thus, transducer prototype 2.1, with a perpendicular outlet of the coupling axis, was investigated. This construction, however, reduced the surgeon's view significantly, risking damage of the implant during implantation. Thus, to decrease the risk of mechanical damage, a protective socket surrounding the coupling axis was developed (Fig 1B). Three different lengths were made. Test implantations showed that there would be enough space for the longest protective socket (Fig 1B; prototype 2.3).

A transducer orientation toward the stapes head or footplate, however, in as many temporal bones as possible, required further minimization of the transducer's shape. As a result, the transducer diameter was reduced from 9.3 mm (Fig 1B) to 8.5 mm (Fig 1C). For technical reasons, a further reduction of the transducer diameter was not possible, so prototype 3.0 (Fig 1C) possessed the smallest transducer diam-

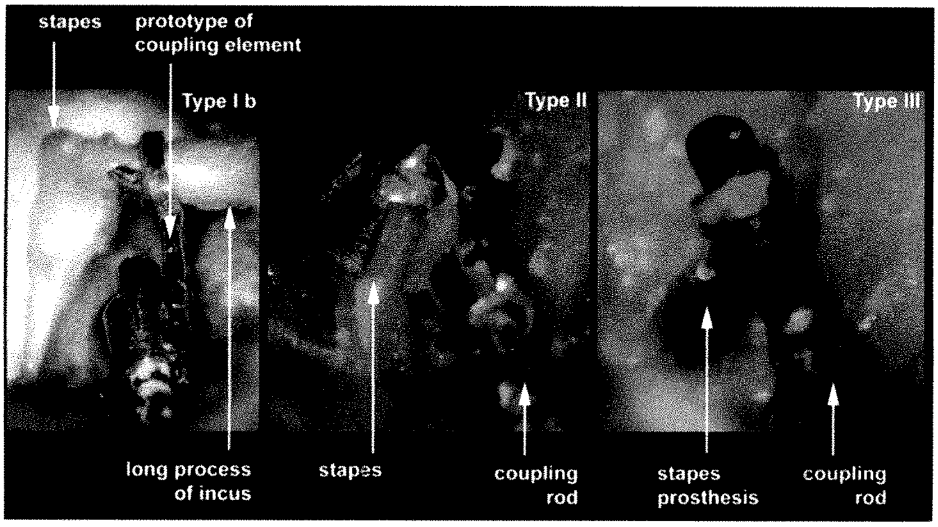

Fig 3. Different types of coupling elements. At left is coupling to long process of incus with loop or clip (type Ib). In middle is coupling to stapes with bell-shaped coupling element (type II). At right is coupling to perilymph with stapes prosthesis used as coupling element (type III).

eter that was technically possible.[3]

Coupling of Transducer. To allow the surgeon to perform exact manipulations during the operation, the final prototype was combined with the coimplantable TICA micromanipulator[11] to allow a secure coupling to the ossicular chain or perilymph (Fig 2). Coupling to the incus body may be useful in patients with mixed hearing loss whose conductive component is a result of destruction of the long process of the incus. In a patient with mixed hearing loss, if reconstruction is done with liquid ionomeric cement or a titanium-angle prosthesis (Plester prosthesis), hearing gain may not be sufficient because of the SNHL component. If the situation does not allow the use of a conventional hearing aid, TICA coupling to the incus body may be indicated in these rare cases. Coupling to the incus body was achieved with a laser, which was used to carve a hole of 500 µm into the ossicle. The transducer axis was easily inserted into this hole and fixed with liquid ionomeric cement, as expected from published clinical data.[5,11] Less rare is the situation of a missing incus (with or without a malleus) with the stapes present. In this case, a bell-shaped coupling element, derived from the Tuebingen titanium prosthesis, may be fixed to the TICA's coupling rod. The bell is put over the stapes head and may move the intact stapes. In the absence of a stapes superstructure, a direct coupling to the perilymph may be performed. For direct coupling to the perilymph in 6 of 50 cases, the coupling axis was inserted into the perilymph through a laser-made perforation in the footplate (Fig 2). In 36 cases, however, the axis reached the oval window at an inappropriate angle, making perilymph coupling impossible. Coupling to the perilymph was achieved with a gold piston that was inserted through the oval window and connected to the coupling rod (Fig 2).

Interestingly, under all of these conditions, a coupling axis length of 20 mm usually allowed all connections, from direct coupling to the perilymph to coupling to the incus body. There was only 1 case in which the coupling axis was too short and required an unusual positioning of the transducer (above the sigmoid sinus). In 7 of the 50 temporal bones, a connection of the coupling axis was not possible, because there was not enough space available for simultaneous placement of the transducer and microphone. Thus, implantation of the final transducer version with a coupling axis of 20 mm was possible in 42 of 50 temporal bone specimens. This outcome leads to an 84% probability of success, with a CLL of 70.9% and a CLU of 92.8%. The probability of success (sensitivity, ie, number of temporal bones correctly predicted as implantable on Schueller x-ray) can be increased to 89.7% (CLL of 75.8% and CLU of 97.1%) by using preoperative imaging techniques to allow patient selection.

DISCUSSION

In the present temporal bone study, possible sites for coupling a piezoelectric transducer to the interrupted ossicular chain were investigated with regard to the transducer's shape. The transducer is part of a modification of the totally implantable hearing device TICA that allows the treatment of middle ear disease. The published TICA version[10] is an implant restricted to the treatment of SNHL. It consists of 4 components: transducer, microphone, amplifier, and energy source.[2-4,12] Its implantation requires the presence of an incus and a stapes. Here, we show that in addition to a linkage to the incus, coupling to the stapes head or even to the perilymph is anatomically and surgically possible. The 3 coupling procedures are as follows.

Type I. If the ossicular chain is intact, coupling of the transducer rod can be performed to the malleus,[13] to the incus body (type Ia),[10] or to the long process of the incus (type Ib; Fig 3). Coupling to the incus body is achieved with a laser[14,15] and/or with ionomeric cement.[16] Coupling Ib requires a posterior tympanotomy and a coupling element that has a loop or a clip.

Type II. If the incus is missing, a connection between the coupling rod and the moveable stapes[10] can be achieved via a bell-shaped coupling element (Figs 2 and 3). Insertion of the coupling axis requires a surgical opening of the facial recess.

Type III. If the stapes superstructure is missing, the vibratory stimulus can be transmitted by direct coupling to the perilymph (Figs 2 and 3). A footplate perforation is performed. A floating footplate can be avoided by using an erbium:YAG laser or a CO_2 laser. To overcome an unwanted angle between the coupling rod and the oval window, a gold stapes prosthesis can be crimped to the coupling rod. As with the type II procedure, a posterior tympanotomy is required. The above-mentioned possibilities of coupling to the ossicular chain are dependent on the specific size of the transducer. In addition, the shape of the implant has a great influence on its performance. Implantability depends on 1) the transducer shape and 2) the patient's individual anatomy. The following technical considerations had a major impact on the final transducer shape: 1) the requirement of a cylindrical shape, 2) a total diameter of 8.5 mm, 3) the mechanical load tolerance at the coupling axis outlet, 4) a coupling axis outlet in the center of the membrane, and 5) a lack of mechanical contact between the transducer and the microphone.

The cylindrical shape was predetermined by the circular oscillating piezoelectric element. This is tightly connected mechanically to a metal plate, forming a heteromorphous element. The diameter of the heteromorphous element could be reduced to 8 mm to allow a first resonance frequency at 10 kHz. Thus, the total diameter of the transducer, including the housing, is 8.5 mm. Anatomically, the individual size and pneumatization of the mastoid bone determine the space available for the implant. Two relevant factors could be elucidated: 1) the distance between the sigmoid sinus and the posterior canal wall and 2) the pneumatization grade, including the root of the zygoma. The final shape of the transducer presented here contributes to both implantability and performance. Furthermore, implantation is facilitated by an appropriate outlet for the cable and a protective socket for the coupling axis. In addition, the socket also serves as a fixation site for a micromanipulator.

The present investigation allowed the use of the TICA implant as a middle ear TICA. The middle ear TICA is thought to contribute in selected patients to the surgical rehabilitation of middle ear malformations and chronic middle ear diseases, such as otosclerosis, that cause mixed hearing losses that cannot be treated with conventional ear surgery or hearing aids. As the TICA contains a microphone that is implanted beneath the skin of the posterior canal wall, the posterior wall of the outer ear canal must be present or reconstructed.

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EFFECT OF INHIBITOR OF TUMOR NECROSIS FACTOR–ALPHA ON EXPERIMENTAL OTITIS MEDIA WITH EFFUSION

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Tumor necrosis factor (TNF)–α is important in the pathogenesis of otitis media with effusion (OME). The purpose of this study was to determine the effect of TNF-α antagonist on the outcome of lipopolysaccharide (LPS)–induced OME in rats. Otitis media was induced by injecting *Pseudomonas aeruginosa* LPS transtympanically. Another (combination) group was pretreated with TNF-α antagonist, soluble TNF receptor type I (sTNF RI), before transtympanic injection of LPS. Saline and phosphate-buffered saline solutions were used as controls. Twelve hours after the transtympanic injection, otoscopic examination and aspiration of middle ear effusion (MEE) were done. The temporal bones in each group were examined histopathologically, and the vascular permeability of the middle ear mucosa was measured by the Evans blue vital dye technique. In the LPS and combination groups, MEE developed in 90% and 0% of ears, respectively. The combination group showed less inflammation, less mucosal thickening, and significantly decreased vascular permeability as compared to the LPS group. Transtympanic administration of sTNF RI appears to suppress the development of LPS-induced OME. This study suggests that TNF-α antagonist, along with antibiotics, may have an adjunctive role in the future treatment of MEE.

KEY WORDS — endotoxin, otitis media with effusion, tumor necrosis factor–alpha antagonist.

INTRODUCTION

Patients with otitis media (OM) may have various other disorders, including mild conductive hearing loss and delays in speech, learning, and behavioral development. Otitis media may also cause permanent middle ear (ME) damage and mucosal changes.[1] In spite of these various sequelae, the pathogenesis of otitis media with effusion (OME) has not been entirely elucidated. The most important cause of acute OM is bacterial entry into the ME. But persistent effusion is found in 10% of children with acute OM, despite prolonged periods (up to 3 months) of medical therapy.[2] According to the worldwide study of Bluestone et al,[3] 34% to 66% of middle ear fluid (MEF) specimens from patients with chronic OME are culture-negative. These facts suggest that other mechanisms play a part in the pathogenesis of chronic OME.

Numerous laboratories have indicated that endotoxin is present in a high percentage of human middle ear effusions (MEEs), including those that are culture-negative.[4] Endotoxin induces an acute inflammatory reaction, which is mediated by various inflammatory mediators.[5-9] Endotoxin and isolated lipopolysaccharide (LPS) have been used to induce experimental OM in various animal species.

Tumor necrosis factor (TNF)–α is a potent proinflammatory agent that plays a pivotal role in a variety of inflammatory reactions. When LPS is injected into the ME, it induces TNF production locally in the ME cleft,[5] which may contribute to the pathogenesis of OM via any number of established inflammatory pathways. Catanzaro et al[10] discovered that recombinant TNF, but not recombinant interleukin-1, produces MEE when injected transtympanically. In an unpublished study, we injected TNF-α transtympanically into the ME of guinea pigs and found that TNF-α can induce MEE.

Soluble TNF receptor type I (sTNF RI) modulates the proinflammatory activity of TNF-α by forming a complex with TNF and blocking the binding of TNF to its cell surface receptors. Therapeutic trials with sTNF RI have been investigated in a number of experiments dealing with other inflammatory diseases.[11]

The purpose of this study was to determine the ef-

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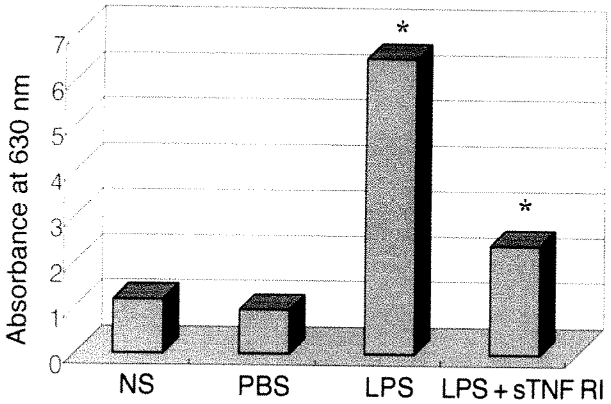

Fig 1. Absorbance of Evans blue dye at 630 nm. Absorbance of lipopolysaccharide (LPS) group was compared with those of control groups — normal saline solution (NS) and phosphate-buffered saline solution (PBS) groups — and showed significant difference. Absorbance for combination group — LPS plus soluble tumor necrosis factor receptor type I (sTNF RI) — was compared with that of LPS group, and it was significantly lower. Asterisks designate statistically significant difference.

fects of sTNF RI on the effusion production, vascular permeability (VP), and histopathologic changes of the ME mucosa in an experimental model of LPS-induced OME.

MATERIALS AND METHODS

The LPS used for this study was derived from *Pseudomonas aeruginosa* (L-9143, Sigma, St Louis, Missouri). It was dissolved in normal saline solution as 1 mg/mL. A TNF antagonist, sTNF RI (PF 055, Oncogene, Cambridge, Massachusetts), was dissolved in phosphate-buffered saline solution at 0.1 μg/mL.

A total of 16 healthy Sprague-Dawley rats weighing 100 to 150 g, free of ME disease as determined with an operating microscope, were used for this study and randomly divided into 2 control groups and 2 experimental groups. All the experiments were performed under ketamine hydrochloride (40 mg/kg) and xylazine hydrochloride (2 mg/kg) anesthesia. The 2 control groups received intratympanic injections of 40 μL of normal saline solution (group A) and phosphate-buffered saline solution (group B), respectively. Forty microliters of LPS was inoculated into the ME of 1 experimental group (group C), and 40 μL of LPS with 10 μL of sTNF RI was transtympanically injected into the other experimental group (combination group; group D) under an operating microscope.

Any MEE present at the end of 12 hours was aspirated. Then, Evans blue dye at a dose of 20 mg/mL per kilogram of body weight was injected into the femoral vein. Thirty minutes after the dye injection, the vascular system was washed out by exsanguination and perfusion of 100 mL of normal saline solution through an intra-aortic catheter. Then, each ME cavity was lavaged with 100 μL of formamide for 5 minutes to collect the extravasated Evans blue dye. The absorbance of each collected sample was measured at a wavelength of 630 nm to determine the degree of dye extravasation with a spectrophotometer.

After the ME lavage, the temporal bones were removed, fixed in 4% paraformaldehyde, decalcified in ethylenediaminetetraacetic acid for 2 weeks, dehydrated in a graded series of alcohols, embedded in paraffin, sectioned to a thickness of 15 μm, and stained with hematoxylin-eosin for microscopic examination. The thickness and inflammatory cell infiltration of the ME mucosa were examined at the thickest region of the promontory mucosa in 3 contiguous sections. The numbers of infiltrated leukocytes and the thickness of the subepithelial layer were measured with a lattice (10 × 10 cells; 25 × 25 μm per cell) under a ×400 high-power field (HPF). The mean count in each group was analyzed with a 2-tailed Student's *t*-test; a p value of less than .05 was considered statistically significant.

RESULTS

Middle ear fluid developed in 1 of 6 ears (16.7%) in group A and in 9 of 10 ears in group C. Middle ear fluid did not develop in groups B and D.

In the control groups, the mean absorbances (±SD) of Evans blue dye at 630 nm were 1.35 ± 0.49 (group A) and 0.96 ± 0.58 (group B). The mean absorbance of the group exposed to LPS alone (group C) was 6.49 ± 3.71, and it was significantly greater than those of the other groups ($p < .001$). The mean absorbance of the combination group (group D) was 1.62 ± 0.39, and it was significantly lower than that of group C ($p < .0005$; Fig 1).

The thicknesses of the ME mucosal layer (Fig 2) for the control groups were 0.02 ± 0.01 mm (group A) and 0.03 ± 0.03 mm (group B). For the ears exposed to LPS alone (group C), the thickness was 0.06 ± 0.03 mm, which was significantly greater than the values for the control groups ($p < .002$). The thickness for the combination group (group D) was lower than that for group C (0.04 ± 0.03 mm), but it was not significantly different (Fig 3).

The mean numbers of leukocytes (cells per lattice) counted in the control groups were 2.34 ± 2.11 (group A) and 4.05 ± 3.36 (group B). For the ears exposed to LPS alone (group C), the mean number was 6.23 ± 3.59, and it was significantly greater than the num-

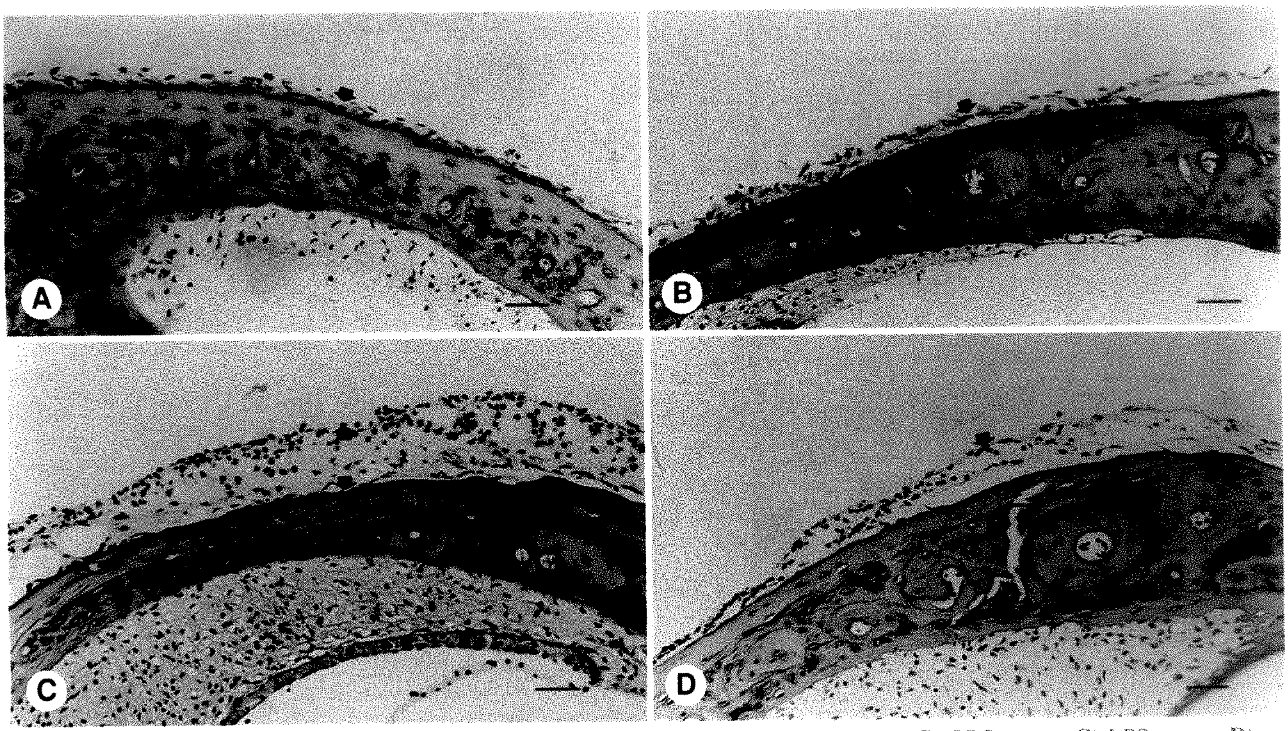

Fig 2. Photomicrographs of promontory mucosa (H & E, original ×400). **A)** NS group. **B)** PBS group. **C)** LPS group. **D)** Combination group. In control groups (NS and PBS), subepithelial layer is very thin and has few leukocytes. In LPS group, subepithelial layer is markedly thickened and infiltrated with many leukocytes. In combination group, subepithelial thickening and number of infiltrated leukocytes are decreased. Arrow — middle ear mucosa; bar — 0.1 mm.

bers for the control groups ($p < .02$). The mean number of the combination group (group D) was 4.56 ± 2.60, ie, lower than that of group C, but the decrement was not significant (Fig 4).

DISCUSSION

When the cause of OM is a gram-negative organism, endotoxin may be trapped in the ME cleft after the microbial infection is controlled with antibiotics. Endotoxin is a collection of gram-negative bacterial cell wall components, and LPS is the biologically active moiety of endotoxin. Many studies have confirmed the presence of endotoxin in the MEF of patients with OME,[4] and endotoxin has been used to induce experimental OM.[5-7] Besides endotoxin, various inflammatory mediators (vasoactive amines, plasma proteases, arachidonic acid metabolites, platelet activating factor, cytokines, free radicals, and lysosomal enzymes) have been detected in MEFs of patients with OME[5,12,13] and have been investigated for their role in the pathogenesis of OM. Platelet activating factor exerts its effect in the pathogenesis of OM by increasing the VP and the secretion of mucous glycoprotein and by impairing the mucociliary function of the eustachian tube.[14-17] Intratympanic injection of IL-2, IL-8, leukotriene C4, or prostaglandin E2 has been shown to induce MEE.[10,18,19] Intravenous injection of bradykinin, histamine, serotonin, acetylcholine, substance P, and vasoactive intestinal polypeptide induces MEE by increasing VP.[20] Thromboxane inhibitor was effective in moderating OME development in a model in which OM was induced by inoculation of live *Streptococcus pneumoniae*.[21] Nitric oxide plays the role of an important mediator in the development of OM by increasing VP and mucin secretion.[9,22] Leukotriene antagonist inhibited OM development in a model of OM induced by killed bacterial inoculation.[23,24]

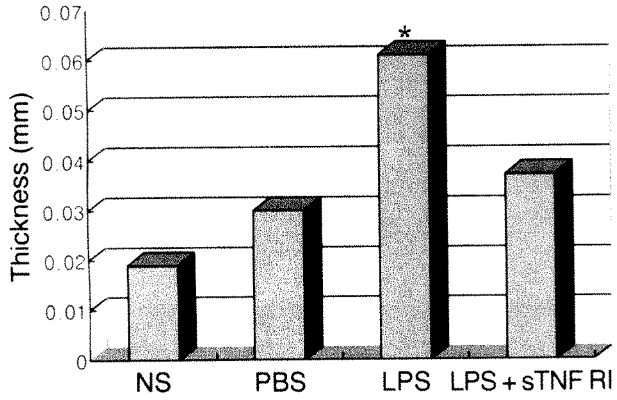

Fig 3. Mean value of subepithelial thickness of promontory mucosa. Subepithelial layer of LPS group was significantly thicker than those of control groups (NS and PBS groups). Subepithelial thickness of combination group was thinner than that of LPS group, but was not significantly different. Asterisk — statistically significant difference.

To sum up these reviews, we may presume that

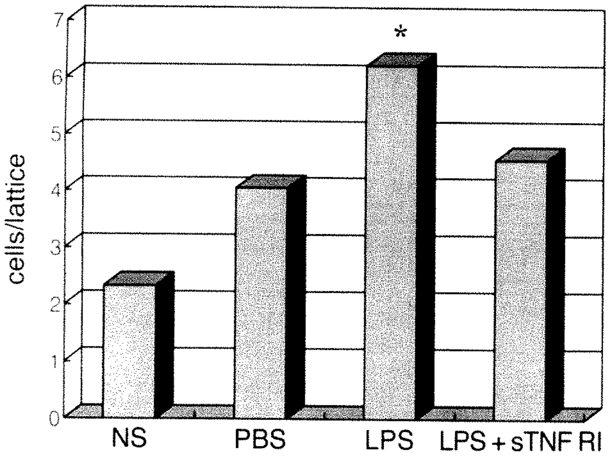

Fig 4. Average count (cells per lattice) of inflammatory cells infiltrated in subepithelial layer of promontory mucosa. Infiltrated number of leukocytes for LPS group showed significant difference from those of NS and PBS groups. Subepithelial leukocyte count of combination group was lower than that of LPS group, but it was not significantly different. Asterisk — statistically significant difference.

chronic OME is initiated with bacterial infection, and that bacterial endotoxin may remain in the ME cleft despite antibiotic therapy, the ME clearance mechanism, and host defense mechanisms. Remaining endotoxin perpetuates a vicious circle of inflammation by stimulating the production of various inflammatory mediators.

Lipopolysaccharide-induced effusion is considered to be the result of microvascular leakage in postcapillary venules. This action is known to be mediated by various inflammatory mediators such as TNF-α,[5,7] IL-1,[8] nitric oxide,[9] and arachidonic acid metabolites.[6] Many studies have demonstrated that LPS-induced vascular extravasation is primarily mediated by TNF-α.[7,25]

Tumor necrosis factor–α is the inflammatory mediator that has been consistently found in human MEEs. Reported levels of TNF-α in human MEEs range widely, from 1.24 to 700 pg/mg total protein of MEF.[8,12,13,26,27] Tumor necrosis factor–α is secreted by macrophages in response to microbial or endotoxin stimulation and contributes to the acute phase of inflammatory responses by acting as a potent proinflammatory agent. Tumor necrosis factor–α increases vascular permeability and vasodilation, activates neutrophils, induces adhesion molecules, promotes the release of other cytokines (IL-1, IL-2, IL-6, and IL-8), and stimulates arachidonic acid metabolism.

Killed or live bacteria inoculated into the ME induces OME, and TNF-α has been detected in the resulting MEFs.[28-30] Intratympanic injection of LPS induces TNF production locally in the ME cleft, and treatment with TNF binding protein moderates the development of OME by decreasing VP.[5,7] Intratympanic injection of TNF-α produces OME, also.[10]

Maxwell et al[27] found a statistically significant relationship between TNF-α and sTNF RI in the MEFs of patients with OME. They suggested that cytokines and their inhibitors seem to be important in the regulation of inflammatory processes in chronic OME. In our experiment, sTNF RI blocked the development of MEE effectively. The gross presence of MEE was identified in 90% (9 of 10 ears) of the LPS group, but there was no effusion in the combination group. Absorbance of Evans blue dye in the MEF, which is an indirect method of measuring VP, also revealed that sTNF RI treatment significantly blocked the increase of VP. This finding coincides with that of Ball et al[7] and further clarifies the role of TNF-α in the pathogenesis of OME.

The thickness of the ME mucosa, which is another indicator of a change in VP, was increased significantly in the LPS group as compared to the control groups. The thickness was decreased in the combination group as compared to the LPS group, but this decrement was not significantly different. Both IL-1 and TNF stimulate neutrophil recruitment. Ball et al[7] demonstrated that sTNF RI suppresses neutrophil recruitment and suggested that neutrophil migration is likely involved. In our experiment, exposure of the ME to LPS induced significantly increased inflammatory cell infiltrates (6.23 cells per lattice, ×400 HPF). Co-treatment with sTNF RI reduced the inflammatory cell infiltrate (4.41 cells per lattice, ×400 HPF), but this decrement was not statistically significant. This finding suggested that mediators other than TNF might contribute to LPS-induced leukocyte infiltration.

We conclude that TNF-α plays an important role in the pathogenesis of OME by increasing the VP of the ME mucosa. Tumor necrosis factor–α antagonist effectively prevents the extravasation induced by LPS in the ME of rats. We suggest that this antagonist, along with antibiotics, may have an adjunctive role in the future treatment of MEE. However, as various inflammatory mediators are involved in the pathogenesis of OME, the most effective treatment strategy for OM would be a general blockade of the cascade of inflammatory responses. As the acute inflammatory response is important in the host defense mechanism, the balance between the inflammatory reaction and its control should be considered. Also, the optimal time to adjust this biological response should be determined.

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HISTOPATHOLOGIC STUDY OF TEMPORAL BONE AND EUSTACHIAN TUBE IN OCULOAURICULOVERTEBRAL SPECTRUM

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Bilateral temporal bone specimens from a 21-month-old girl and a left temporal bone–eustachian tube (ET) specimen from a full-term female newborn, both with oculoauriculovertebral spectrum, were studied histopathologically. The external and middle ears demonstrated severe anomalies, similar to those of previous reports describing the histopathologic findings of this syndrome. In addition, despite having a normal auricle, the 21-month-old child had bilateral hypoplastic cochleas as seen in Mondini dysplasia. The newborn had several anomalies of the ET, including a widely opened cartilaginous portion of the ET lumen and absence of the lateral lamina of the ET cartilage. We discuss the implications of the observed anomalies with regard to developmental and clinical issues.

KEY WORDS — anomaly, eustachian tube, histopathology, oculoauriculovertebral spectrum, temporal bone.

INTRODUCTION

The term oculoauriculovertebral dysplasia was first proposed by Gorlin et al[1] to describe patients with epibulbar dermoids or lipodermoids, microtia, mandibular hypoplasia, and vertebral anomalies. The anomalies identified with oculoauriculovertebral dysplasia, including hemifacial microsomia and classic Goldenhar's syndrome, are considered to be congenital malformations secondary to developmental abnormalities of mostly first and second branchial arch derivatives.[2] Cohen et al[3] suggested the term oculoauriculovertebral spectrum (OAVS) to describe these conditions. The location and degree of anomalies of the ear recognized in OAVS vary in each case. There are numerous reports describing developmental anomalies affecting the ear, mandible, eye, vertebrae, skeleton, and central nervous and cardiovascular systems.[2-5] There is limited information describing histopathologic abnormalities of the temporal bone in OAVS,[4,6-8] and there has not been a histopathologic report describing the middle ear (ME) and inner ear (IE) abnormalities recognized in the contralateral temporal bone. Furthermore, the microscopic findings of the entire eustachian tube (ET) and its associated structures have not been recorded. The purpose of this report is to present and discuss the results of histopathologic examination of bilateral temporal bone specimens from one case of OAVS and a temporal bone–ET specimen from another case.

CASE REPORTS

Case 1. A 21-month-old girl died of increasing intracranial pressure and recurrent respiratory distress. She had hydrocephalus treated by a ventriculoperitoneal shunt, asymmetric micrognathia (severe on left side), aplasia of the left external ear, cleft palate, and a dermoid of the skull treated by surgical excision. No vestibuloauditory function testing was performed. The following abnormalities and anomalies were noted during the autopsy: 1) of the skull, macrocephaly; 2) of the brain, Dandy-Walker malformation with hydrocephalus, hypoplastic optic nerve and chiasm (bilateral), hypoplastic choroid plexuses, agenesis of the corpus callosum, and subacute leptomeningitis and choroiditis; 3) of the eye, cataracts (bilateral) and a hypoplastic optic nerve (left more severe than the right); 4) of the lung, acute pneumonia (bilateral) and fibrous adhesions of the right lung to the parietal pleura; and 5) of the abdomen, cyst formation connecting to the distal orifice of the ventriculoperitoneal shunt. In this patient, a chromosome analysis confirmed a normal female karyotype 46 XX.

Both temporal bones were obtained 8 hours after death. After fixation in 10% formalin, decalcification in 5% trichloroacetic acid, and dehydration in graded solutions of alcohol, the specimens were embedded in celloidin and sectioned horizontally at 20 µm. Every 10th section was stained with hematoxylin and eosin and examined under a light microscope.

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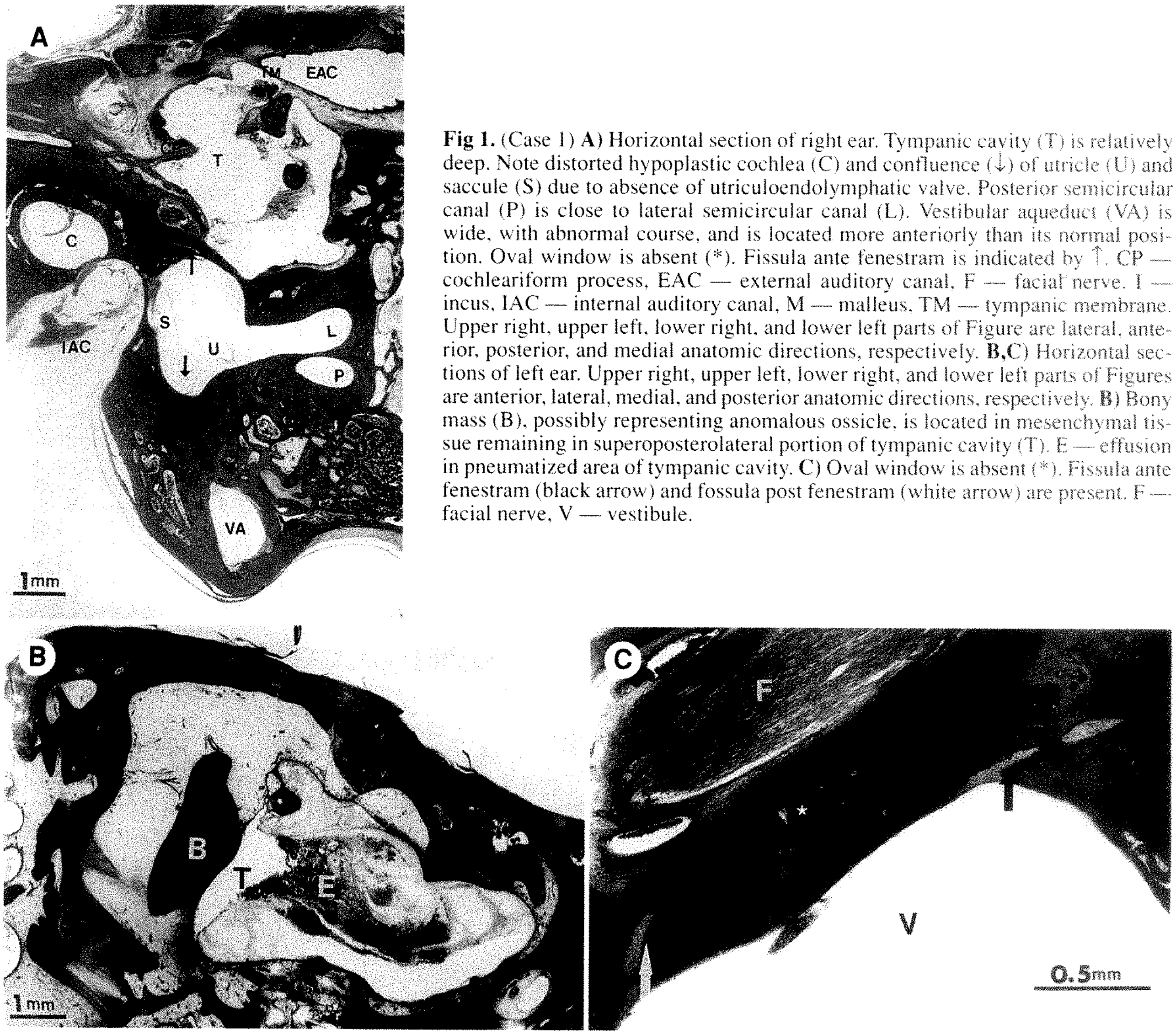

Fig 1. (Case 1) **A)** Horizontal section of right ear. Tympanic cavity (T) is relatively deep. Note distorted hypoplastic cochlea (C) and confluence (↓) of utricle (U) and saccule (S) due to absence of utriculoendolymphatic valve. Posterior semicircular canal (P) is close to lateral semicircular canal (L). Vestibular aqueduct (VA) is wide, with abnormal course, and is located more anteriorly than its normal position. Oval window is absent (*). Fissula ante fenestram is indicated by ↑. CP — cochleariform process, EAC — external auditory canal, F — facial nerve, I — incus, IAC — internal auditory canal, M — malleus, TM — tympanic membrane. Upper right, upper left, lower right, and lower left parts of Figure are lateral, anterior, posterior, and medial anatomic directions, respectively. **B,C)** Horizontal sections of left ear. Upper right, upper left, lower right, and lower left parts of Figures are anterior, lateral, medial, and posterior anatomic directions, respectively. **B)** Bony mass (B), possibly representing anomalous ossicle, is located in mesenchymal tissue remaining in superoposterolateral portion of tympanic cavity (T). E — effusion in pneumatized area of tympanic cavity. **C)** Oval window is absent (*). Fissula ante fenestram (black arrow) and fossula post fenestram (white arrow) are present. F — facial nerve, V — vestibule.

In the right ear, both the external auditory canal and the tympanic membrane appeared to be normal. The tympanic cavity was well pneumatized and relatively deep. An ME effusion with inflammatory cells was present. Submucosal inflammatory cell infiltration was observed in the ME mucosa. A moderate amount of mesenchymal tissue remained, mainly in the epitympanum and antrum. The poorly developed mastoid air cells were filled with effusion and cholesterol granuloma. The malleus had an anomalous manubrium widely attached to the tympanic membrane. The incus was bulky. The stapes was hypoplastic and represented only by its superstructure and was dislocated posteriorly. Both the tensor tympani muscle and the stapedius muscle were present; however, the latter was exposed into the ME cavity through the pyramidal eminence. The facial nerve had an aberrant route, running inferiorly between the round window niche and the sinus tympani. Both the chorda tympani nerve and Jacobson's nerve appeared to be normal. Similarly, the greater superficial petrosal nerve and the lesser superficial petrosal nerve could be identified.

The cochlea was distorted and hypoplastic, with approximately 1 turn (Fig 1A). The organ of Corti appeared to be well developed; however, it was difficult to evaluate further because of the presence of postmortem changes. The cochlear nerve, spiral ganglion cells, and cochlear aqueduct were noted to be within normal limits. In the vestibular system, because of the absence of the utriculoendolymphatic valve, confluence of the utricle and saccule was observed (Fig 1A). The posterior semicircular canal was very close to the nonampullated end of the lateral semicircular canal; otherwise, the semicircular canals were normal. The vestibular end organs, vestibular nerves, and Scarpa's ganglion cells appeared to be well developed and normal. However, the endolymphatic sac and vestibular aqueduct were wide, with an abnormal route, running more anteromedially than the normal course (Fig 1A). The oval window was absent, and there was no connection between the anomalous stapes and the otic capsule. The round window appeared to be normal. The internal auditory canal was slightly wide. The facial, vestibular, and cochlear nerves all appeared to be within normal limits.

In the left ear, atresia of the external auditory canal was observed. The tympanic membrane was absent. The tympanic cavity was severely distorted and was filled with a large amount of mesenchymal tissue in its epitympanum. The mastoid antrum and air cells were also poorly developed. An effusion and inflammatory cell infiltration were present in the tympanic cavity and mastoid. The ossicles were not identified. Instead, a bony mass was present in the mesenchymal tissue in the superolateral portion of the tympanic cavity (Fig 1B). Neither the tensor tympani muscle nor the stapedius muscle could be identified. The facial nerve had an abnormal route, running more laterally than its normal course. The chorda tympani nerve could not be identified; however, Jacobson's nerve was observed. The greater superficial petrosal nerve and lesser superficial petrosal nerve were also identified and appeared to be within normal limits.

In the IE, the findings were similar to those in the right temporal bone, except that the cochlear aqueduct was not identified, despite the presence of the inferior cochlear vein. In the vestibular system, the utricle, saccule, and vestibule all appeared to be normal. The oval window, again, was absent (Fig 1C). The internal auditory canal was relatively narrow. The facial, vestibular, and cochlear nerves were all within normal limits.

Case 2. A full-term boy, weighing 2,700 g at birth, was delivered by cesarean section to a 33-year-old gravida IV para III mother. His Apgar score was 7, and he died of respiratory distress 2 days after birth. He had multiple anomalies. At the autopsy, the following abnormalities and anomalies were noted: 1) a small and asymmetric face with a cleft lip and microtia with bilateral preauricular ear tags (left side more abnormal than right); 2) of the brain, cerebellar hypoplasia, asymmetric hippocampal hypoplasia, dysgenesis of olfactory bulb and tract; 3) of the vertebrae, cervical and upper thoracic vertebral body hypoplasia; 4) of the limbs, radial aplasia (left) and absent thumb (left); and 5) of the heart, an atrial septal defect, a large ductus arteriosus, a ventricular septal defect, an interrupted aortic arch and aortic stenosis, and a double-outlet right ventricle. In this patient, a chromosome analysis confirmed a normal male karyotype 46 XY.

The left temporal bone with both the ET and its associated structures was obtained 18 hours after death. After being processed by the routine method mentioned above, the specimen was sectioned vertically in a plane perpendicular to the long axis of the ET at 20 µm.[9] Every 20th section was stained with hematoxylin and eosin and examined under a light microscope.

The external auditory canal was narrow and poorly developed. A normal tympanic membrane was not present. The early formation of the tympanic membrane was composed of a dense layer of connective tissue, lined by the medial extension of the epidermal layer of the external auditory meatus and the lateral extension of the mucosal layer of the tympanic cavity (Fig 2A). The poorly developed tympanic cavity was filled with mesenchymal tissue except for a very small space connecting to the tympanic orifice of the ET. A large amount of mesenchymal tissue remained in the antrum and mastoid.

The malleus and incus were so malformed that their exact identification could not be confirmed. Two bony masses presumed to be the malleus and incus were located in the mesenchymal tissue in the epitympanic cavity (Fig 2B). A strand of cartilaginous tissue, running inferomedially from the bony mass presumed to be the deformed body of the incus, was suggestive of the long process of the incus. The stapes was absent. The oval window niche was very narrow and deep; at its most medial extent, a small cartilaginous portion of the otic capsule, which probably was the primitive oval window, was observed (Fig 2C).

The tensor tympani muscle was well developed; however, its tendon was not inserted into the bone presumed to be the malleus. Similarly, the stapedius muscle, without its tendon, could be identified. The facial nerve was located in an inferoanteriorly dislocated position. Although the greater superficial petrosal nerve, the lesser superficial petrosal nerve, and Jacobson's nerve were present, the chorda tympani nerve could not be identified. There were no inflammatory findings within the ME.

Near the pharyngeal orifice of the ET, the lumen was moderately wide and patent. Both the torus tubarius and Rosenmuller's fossa were poorly developed (Fig 2D). In the cartilaginous portion of the ET, the lumen was also wide and open. There were a few ridges of mucosal folds along the floor of the ET lumen. The ET cartilage was anomalous. The lateral lamina (LL) of the ET cartilage was severely undeveloped and almost absent. The medial lamina (ML) of the ET cartilage was dislocated laterally with a counterclockwise rotation, lying between the ET

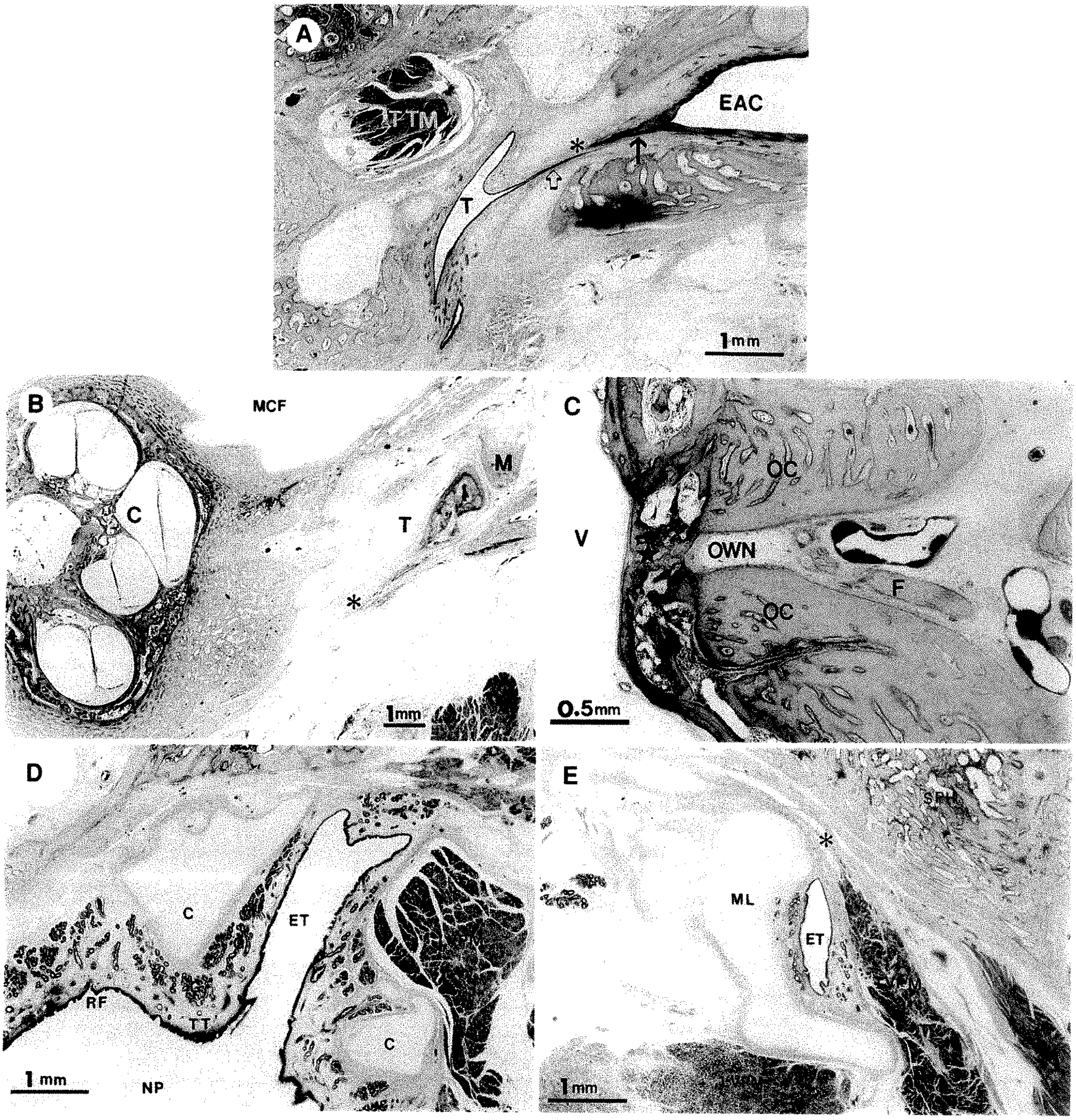

Fig 2. (Case 2) Vertical sections of left ear. Right, left, superior, and inferior parts of Figures are anterolateral, posteromedial, superior, and inferior anatomic directions, respectively. **A)** External auditory canal (EAC) is narrow. Note primordial tympanic membrane (*) and poorly developed tympanic cavity (T). Epidermal layer (black arrow) of EAC and mucosal layer (white arrow) of T start to form primitive tympanic membrane. Tensor tympani muscle (TTM) is well developed. **B)** Two bony masses, presumed to be malleus (M) and incus (I), are observed in mesenchymal tissue in superolateral portion of tympanic cavity (T). Strand of cartilaginous tissue (*), running inferomedially, is probably long process of incus attaching to rudimentary body of incus. Cochlea (C) is normally developed. MCF — middle cranial fossa. **C)** Very narrow, deep oval window niche (OWN) and small amount of cartilaginous tissue (star), which probably is primitive oval window, are observed. Otic capsule (OC) is very thick. F — facial nerve, V — vestibule. **D)** Pharyngeal orifice of eustachian tube (ET) is relatively wide and patent. Torus tubarius (TT) and Rosenmuller's fossa (RF) are poorly developed. C — ET cartilage, G — tubal glands, NP — nasopharynx, LVPM — levator veli palatini muscle. **E)** Midcartilaginous portion of ET demonstrates patent ET lumen, rather than normal slitlike luminal space. Lateral lamina of ET cartilage is absent (*). Medial lamina (ML) of ET cartilage shows its counterclockwise rotation, intervening between ET lumen above and LVPM below. Tensor veli palatini muscle (TVPM) is not attached to ET cartilage. Ostmann's fatty tissue is also undeveloped. SPH — sphenoid bone.

lumen above and the levator veli palatini muscle (LVPM) below (Fig 2E). Attachment of the tensor veli palatini muscle (TVPM) to the ET cartilage was not observed. The ET glands were well developed.

However, Ostmann's fatty tissue was undeveloped. In contrast, the ET lumen was rather narrow, with a slitlike shape in the junctional[10] and bony portions of the ET. As mentioned above, the narrowed ET then communicated with the very small tympanic cavity. There were no inflammatory findings within the ET.

The cochlea was normally developed, with 2$^3/_4$ turns (Fig 2B). The organ of Corti appeared to be well developed; however, it could not be further analyzed because of the presence of postmortem changes. The cochlear aqueduct, cochlear nerve, and spiral ganglion cells all appeared to be normal. The round window was normal. In the vestibular system, the utricle, saccule, vestibule, 3 semicircular canals, endolymphatic sac, and vestibular aqueduct, as well as the vestibular nerves and Scarpa's ganglion cells, were also well developed and normal. The internal auditory canal was slightly shortened and widened. The facial, vestibular, and cochlear nerves were all present and appeared to be normal.

DISCUSSION

Although OAVS is a commonly accepted pathological condition, there remains little agreement in regard to nosology or even minimal diagnostic criteria.[2,3] Moreover, as many other terms, such as hemifacial microsomia, first and second branchial arch syndrome, facio-auriculo-vertebral malformation spectrum, and Goldenhar's syndrome, have been used to describe these congenital anomalies,[3] there is a great deal of confusion regarding this entity. Furthermore, OAVS has heterogeneous causes and pathogenicities.[3] Although the majority of OAVS cases are presumed to occur sporadically,[3,4,6,7] vascular insufficiencies and abnormalities are considered to be potential pathogenic factors that cause this syndrome.[1,11] Oculoauriculovertebral spectrum has a broad range of phenotypic expression.[2,3] Cases do not necessarily manifest all of the major and minor abnormalities. Despite the absence of some features in our cases (vertebral manifestations in case 1 and eye manifestations in case 2), the described findings seem to be compatible with the diagnosis of OAVS.

The abnormal temporal bone findings in these 2 cases can be categorized and summarized as follows. Case 1 showed atresia of the external auditory canal (left ear), distorted ME cavities (both ears), anomalous ossicles (both ears), absence of the stapes (left ear), an abnormal course of the facial nerve (both ears), absence of both the stapedius muscle and the tensor tympani muscle (left ear), absence of the oval window (both ears), hypoplastic cochleas (both ears), anomalously dislocated endolymphatic sacs and vestibular aqueducts (both ears), dislocated posterior semicircular canals (both ears), and otitis media with effusion associated with cholesterol granuloma (both ears).

Case 2 showed a hypoplastic external auditory canal, an undeveloped tympanic membrane, a hypoplastic ME cavity and mastoid, an anomalous malleus and incus, absence of the stapes, an abnormal course of the facial nerve, an anomalous ET, ET cartilage, and TVPM, and absence of the oval window.

This report, to our knowledge, is the first to describe the histopathologic findings of the entire ET and its associated structures in a patient with OAVS. The ET is derived from the expanding terminal end of the endoderm-lined first pharyngeal pouch and probably the second pharyngeal pouch.[12] During the development of the ET in fetal life, the cartilaginous portion of the ET lumen becomes slitlike[13] during the 16th week. The ET cartilage attains its final form with the LL and ML during the 20th week.[14] Accordingly, the maldevelopment of the ET lumen and cartilage observed in case 2 may be explained by an interruption that occurred before the 16th gestational week, because of the presence of an oval ET lumen and the absence of the LL of the ET cartilage. Moreover, since other anomalies, including the absence of attachment of the TVPM to the ET cartilage and an abnormal rotation of the L-shaped ML, were also observed in case 2, we hypothesize that the ET function in this case would be poor. Therefore, if this infant had survived through infancy with the ET structural anomalies described above, he would have likely suffered from otitis media with effusion, as did the patient in case 1.

The ME anomalies described in the present 2 cases were considerably similar to those of previous histopathologic reports.[4,7] Anomalies involving the ME of affected ears in cases of OAVS commonly involve such structures as the ossicles, stapedius muscle, and facial nerve canal. These are derived from the mesoderm of the first and/or second branchial arches.[12] An asymmetric appearance of the ear and face is one of the characteristic symptoms of OAVS. With regard to the ear, it is considered that approximately two thirds of OAVS patients have unilateral microtia.[2] However, in case 1, both the contralateral ear, with a nearly normal appearance of the auricle and external auditory canal, and the affected ear, with prominent, severe anomalies including auricular atresia, had several ME anomalies. Accordingly, our findings suggest that clinicians who care for patients with OAVS should carefully evaluate the contralateral ear, even if it has an almost normal appearance. In addition, since severe anomalies of the oval window and stapes were observed in the present study, it is assumed that patients with OAVS might have significant conductive hearing loss. An otologist who is contemplating repair of the ossicular chain should be aware of the profound abnormalities potentially present in patients with OAVS. Thin-cut, bone-windowed CT imaging should be performed.

The IE is derived from the otic placode that invaginates from the surface ectoderm of the embryo in early fetal life.[12] It has been suggested that OAVS is closely related to abnormalities of the first and second branchial arches, since the IE is not necessarily involved. In previous histopathologic reports of OAVS, however, several IE anomalies were presented.[6-8] Furthermore, in our case 1, severe IE anomalies of the Mondini type[15] were observed in both ears. Although the precise pathogenesis of IE involvement in OAVS is not known, we speculate that the severity of the vascular insufficiency, presumed to be a causative factor of OAVS,[1,11] occurs early in fetal development. The IE involvement as manifested by a hypoplastic cochlea (case 1) likely occurs earlier than the 9th gestational week, because the cochlear duct has the full complement of 2¾ turns by the end of the 8th gestational week.[12]

Otologists should be aware that surgical correction of a conductive hearing loss attributable to anomalies of the ME and/or external auditory meatus in patients with OAVS may be hampered by other congenital anomalies. The potentially aberrant course of the facial nerve must be carefully investigated. Furthermore, Mondini-type IE anomalies may manifest themselves in sensorineural hearing loss and significant abnormalities of the stapes and oval window niche. Careful audiological and imaging studies should be performed to estimate the presence and degree of IE involvement. Severe abnormalities of the ME and otic capsule may warrant consideration of the use of a bone-anchored hearing aid,[16] if an external auditory canal cannot be constructed.

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TRANSIENT INFLAMMATION AND DYSFUNCTION OF THE EUSTACHIAN TUBE SECONDARY TO MULTIPLE EXPOSURES OF SIMULATED GASTROESOPHAGEAL REFLUXANT

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Gastroesophageal reflux is a common problem in the newborn and preschool periods. Recent research suggests that it may be related to eustachian tube dysfunction and otitis media with effusion. The purpose of this experiment was to investigate the relationship between simulated gastroesophageal reflux and eustachian tube dysfunction. Rat middle ears were repeatedly exposed (transtympanically) to pepsin in hydrochloric acid or to phosphate-buffered saline solution. Their eustachian tube function was evaluated by assessing passive opening and passive closing pressures, and active clearance of positive and negative pressure. The passive pressure function tests showed variable results. The rats exposed to pepsin in hydrochloric acid had an impaired ability to clear positive and negative pressure from the middle ear as compared to the rats exposed only to phosphate-buffered saline solution. The results demonstrate that multiple middle ear exposures to pepsin in hydrochloric acid leads to eustachian tube dysfunction in rats.

KEY WORDS — eustachian tube dysfunction, gastroesophageal reflux, middle ear effusion, otitis media, otitis media with effusion.

INTRODUCTION

Gastroesophageal reflux (GER) in pediatric patients is often perceived as a developmental disorder. It is a common complaint in the first few months of life, with significant resolution as the child makes the transition to a solid diet. A subset of children have persistent GER into their preschool years and beyond,[1] and these children are more likely to experience long-term problems. As physicians have investigated pediatric GER, the clinical manifestations have become increasingly clear. Gastroesophageal reflux has been identified as a causative factor in subglottic stenosis, chronic cough, croup, and even apnea.[2] This disorder has also been linked to the development of many airway disorders: croup, chronic cough, laryngospasm, laryngomalacia, vocal cord nodules, asthma, reactive bronchoconstriction, apnea, sudden infant death syndrome, rhinitis, sinusitis, subglottic stenosis, and glottic granuloma.[2]

Euler[3] postulated that GER may be involved in the pathogenesis of otitis media (OM). Otitis media is the second most common disease in childhood,[4] and approximately 5 billion dollars per year are spent on the medical and surgical management of this condition.[5] In 1980, the Ad Hoc Committee on Definition and Classification of Otitis Media and Otitis Media With Effusion classified this disease by its chronicity, as well as by the presence or absence of an effusion.[4] Otitis media with effusion (OME) can be further classified as serous, mucoid, or viscous.[4]

The cause of OME has been thought to be related to the anatomic differences between the adult and pediatric eustachian tubes (ETs).[6] Nonetheless, research has identified other factors, such as viral upper respiratory tract infection, allergy, and ciliary dysfunction, as likely causes of OME.[5] Bluestone[7] states that eustachian tube dysfunction (ETD) is the most important factor in the pathogenesis of middle ear (ME) disease and OME.

An increasingly important issue in pediatric oto-

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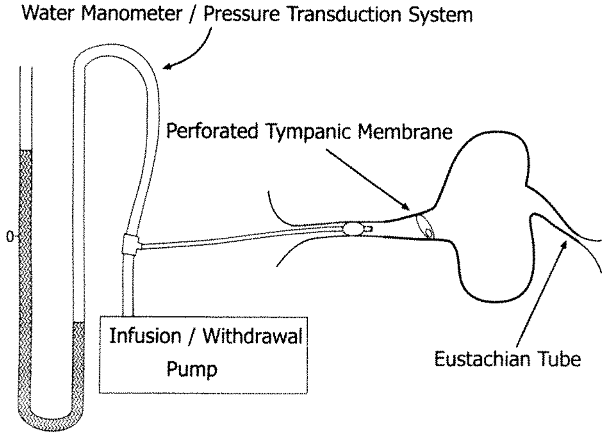
Water Manometer / Pressure Transduction System
Perforated Tympanic Membrane
0
Infusion / Withdrawal Pump
Eustachian Tube

Fig 1. Pressure system described in Materials and Methods. Air moves between infusion-withdrawal pump and middle ear; pressure changes are measured on water manometer. Rat ear has shorter external canal than does human ear, and more superior eustachian tube orifice.[15,16]

laryngology is the relationship between OME and GER. Contencin and Narcy[8] first noted this potential relationship when they published a study documenting the presence of gastric juice in the nasopharynx. Contencin et al[9] later postulated that gastric contents in the nasopharynx may lead to inflammatory changes in the ET leading to ETD and OME. In our own laboratory, Butehorn et al[10] experimentally demonstrated this connection in a rat model. After a single ME exposure to pepsin and hydrochloric acid (HCl), the main constituents of gastric juice, they found dysfunctional ET opening pressures. In the present project, we sought to explore this relationship in more detail. We devised a model in which animals were repeatedly exposed to 1 of 2 different concentrations of pepsin-HCl over a period of time.

MATERIALS AND METHODS

Animals. All research was conducted under approval of the Institutional Animal Care and Use Committee at the University of North Carolina at Chapel Hill. The experiments were performed on 19 Sprague-Dawley rats weighing between 240 and 410 g. Food and water were provided freely throughout the study; the rats were weighed every third day of the experiment to ensure adequate nutrition.

Experimental Design. The rats were randomized to 1 of 3 groups during the experiment. Group A (n = 5), the control group, consisted of rats exposed to transtympanic phosphate-buffered saline solution (PBS). Group B (n = 7) consisted of rats exposed transtympanically to 0.5 mg of pepsin (Sigma Chemical Co, St Louis, Missouri) in 1 mL of 0.01N HCl. Group C (n = 7) consisted of rats exposed transtympanically to 2.0 mg of pepsin in 1 mL of 0.01N HCl. All rat MEs were injected with 35 μL of the PBS or pepsin-HCl solution on the basis of pre-experiment measures showing rat ME volumes to be between 39 and 50 μL. The rat MEs in group B were therefore exposed to 0.0175 mg of pepsin in 35 μL HCl, and those in group C were exposed to 0.070 mg of pepsin in 35 μL HCl.

Pepsin was used with 0.01N HCl (pH 2) on the basis of earlier reports citing 1) that pepsin (with HCl) is necessary for significant mucosal damage,[11] and 2) that pepsin is optimally active at pH 2.[12] Regarding the pepsin concentration, most experimental protocols use between 0.3 and 1.0 mg of pepsin per milliliter of HCl to induce mucosal damage; ours conformed with the procedure of Butehorn et al,[10] in which 0.5 mg/mL was used. No experimental models have used a pepsin concentration as high as 2.0 mg/mL, although Ohashi and Nakai[13] labeled this concentration that of "crude gastric juice." We used a PBS of pH 7.4.

Surgical Preparation and Perioperative Management. Before the experimental procedure, each rat was anesthetized with 0.1 mL per 100 g of body weight of a 1:1 mixture of ketamine hydrochloride (100 mg/mL) and xylazine (20 mg/mL). Intravascular volume was maintained during the procedure with subcutaneous injections of 0.9% sodium chloride solution given at 80 μL per kilogram of body weight per minute. The body temperature was maintained at 37°C with a thermostat-controlled heating pad.

Evaluation of Tubal Function. After induction of anesthesia, we placed the rats in the right lateral recumbent position in order to visualize their left tympanic membrane (TM) with the operating microscope. After ruling out preexisting ME disease, we made 2 myringotomies, approximately 0.5 mm in diameter, in the anterior and posterior inferior quadrants of the TM with a 25-gauge needle. One myringotomy served as an injection port, and the other as a vent. Next, the baseline ET function was measured. The ET function was evaluated according to 4 tests: passive opening pressure (POP), passive closing pressure (PCP), active clearance of positive pressure (ACPP), and active clearance of negative pressure (ACNP). The POP and PCP were determined by the forced-response test initially described by Flisberg et al[14] (Fig 1[15,16]). Briefly, an airtight seal was created by inflating the balloon of a 100% silicone 6F pediatric Foley catheter (Rusch Inc, Duluth, Georgia) in the external auditory canal. The drainage lumen of the catheter was connected via a T-valve to an adjacent water manometer and an infusion-withdrawal pump (Harvard Apparatus Co, Inc, Millis, Massachusetts). The infusion-withdrawal pump infused air into the ME at a constant rate of 20 mL/

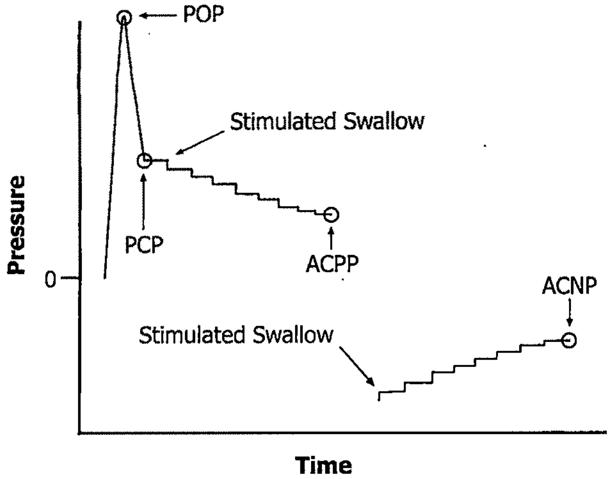

Fig 2. Time-pressure schematic demonstrates pressure changes within each eustachian tube function test session. At passive opening pressure (POP), eustachian tube has opened, and at passive closing pressure (PCP), it has closed. For both positive and negative pressure clearance tests, rats were stimulated to swallow; steplike pattern represents degree of pressure equalization with each swallow. Irregularity of step pattern is indicative of variable pressure changes that occurred with subsequent swallows. Active clearance of positive pressure (ACPP) and active clearance of negative pressure (ACNP) represent levels at which continued swallowing produced no further changes in level of positive pressure or negative pressure, respectively.

min until the ET could no longer maintain a closed system. Upon tubal opening, the water meniscus on the manometer changed from convex to concave, and the pressure read was labeled as the POP (Fig 2). At this point, the infusion was stopped and the water column was allowed to fall. The pressure at which the ET equilibrated and the water column no longer fell was labeled as the PCP (Fig 2).

Evaluation of the ACPP and ACNP required the rats to swallow. According to earlier research, swallowing contracts the tensor veli palatini muscle, the primary muscle involved in opening of the ET.[5] With the closed system at PCP, the rat was stimulated to swallow by placement of a small polyethylene tube in the posterior pharynx; each swallow produced a slight drop in the water column. The point at which the column no longer fell with continued swallowing was identified as the ACPP (Fig 2). Additionally, by means of this closed system, the infusion-withdrawal pump was used to create a negative pressure of −20 cm H_2O in the ME as gauged on the manometer system. Swallowing was also used to clear this pressure. The level at which continued swallowing produced no change in the water column level was identified as the ACNP (Fig 2).

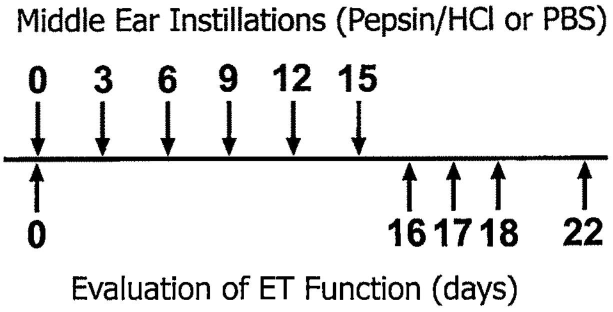
Middle Ear Instillations (Pepsin/HCl or PBS)
0 3 6 9 12 15
0
16 17 18 22
Evaluation of ET Function (days)

Fig 3. Experimental timeline described in Materials and Methods. Rats were transtympanically exposed to pepsin–hydrochloric acid (HCl) or phosphate-buffered saline solution (PBS) on days 0, 3, 6, 9, 12, and 15 of experiment. Eustachian tube function was tested on days 0, 16, 17, 18, and 22 of experiment.

After baseline pressure measurements on day 0 of the experiment, the rats were placed in their respective experimental groups. Each experimental group received transtympanic pepsin-HCl solution or PBS according to the following schedule (Fig 3): day 0 (after baseline pressures), day 3, day 6, day 9, day 12, and day 15. After all exposures, the POP, PCP, ACPP, and ACNP were evaluated 1, 2, 3, and 7 days after the last exposure (ie, day 16, day 17, day 18, and day 22 of the experiment). Before each pressure measurement, the TMs were inspected to rule out evidence of new disease.

Statistical Analysis. The POP, PCP, ACPP, and ACNP values were analyzed with a 2-way analysis of variance with Bonferroni's method of pairwise comparison. A p value of less than .05 was considered a statistically significant change between groups. Bar graphs were made of mean pressure values with SEM versus time for each group.

RESULTS

Passive Opening Pressure. The rats repeatedly exposed to PBS (group A) showed no changes in pressure values over the test period. The rats repeatedly exposed to pepsin-HCl (groups B and C) exhibited results different from those of the control rats in group A (Fig 4A). The rats in group B (0.5 mg of pepsin per milliliter of HCl) showed an increased POP as compared to controls for all days after pepsin-HCl exposure except day 22 (the seventh day after exposure). The overall difference in POP between group B and the control group was significant ($p = .002$). Although the POP levels were increased on days 16, 17 and 18, only on day 18 were the values significantly increased over the baseline POP levels. The day 22 values returned to baseline.

In group C (2.0 mg of pepsin per milliliter of HCl), the POP decreased as compared to controls for all days after exposure (Fig 4A). This overall decrease was significant ($p = .038$). Unlike group B, group C exhibited no time-dependent variations after expo-

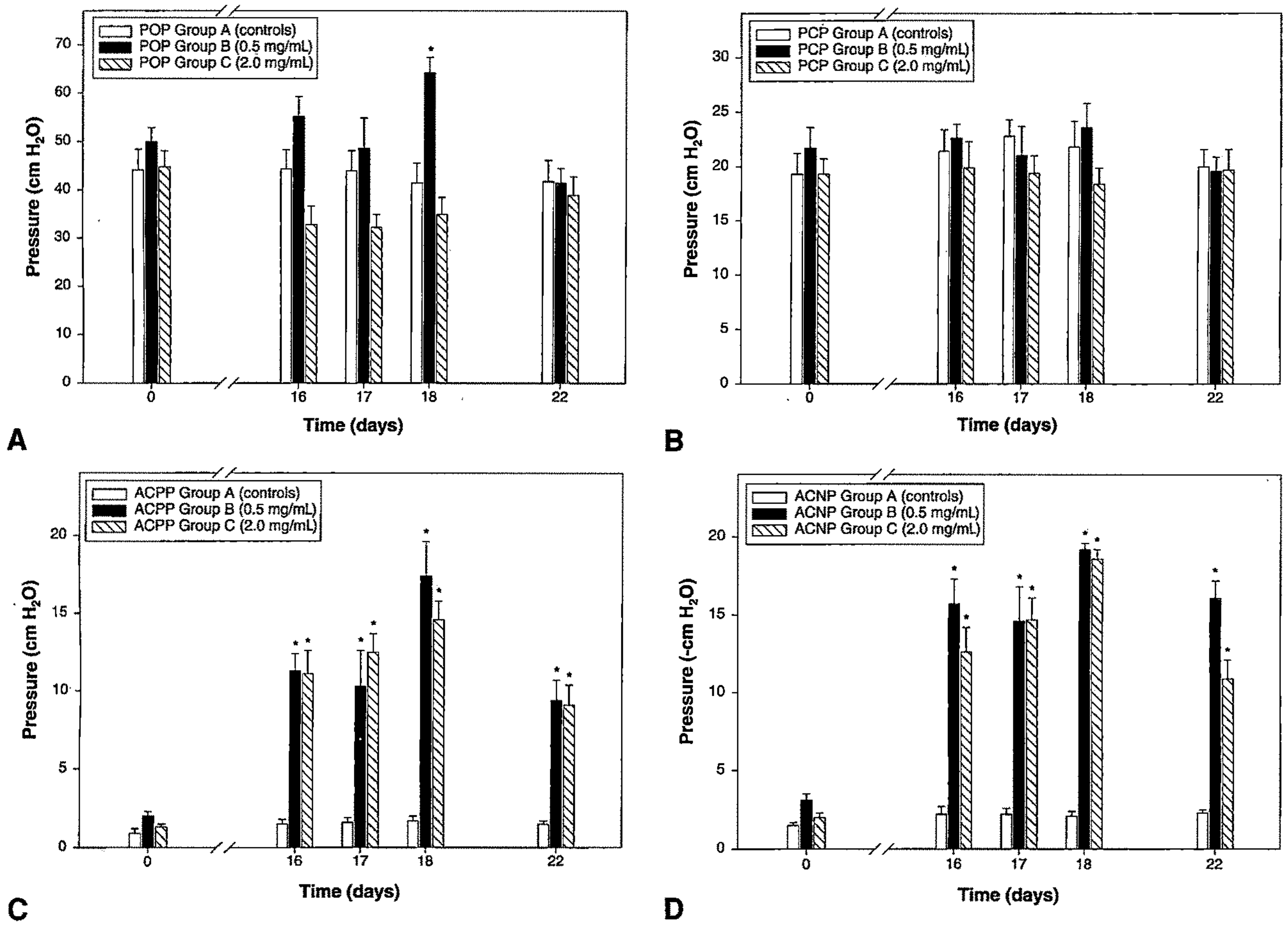

Fig 4. Measurements made on days 0, 16, 17, 18, and 22 of experiment. Note break between days 0 and 16: 6 pepsin-HCl exposures occurred during this period. Error bars represent SEM. Asterisk represents statistically significant increase in pressure on that day over control values. **A**) Passive opening pressure. **B**) Passive closing pressure. **C**) Active clearance of positive pressure. **D**) Active clearance of negative pressure.

sure on days 16, 17, and 18. Once again, day 22 showed an almost complete return to baseline pressures.

There were substantial differences in POP levels between groups B and C. An overall significant difference existed between the two experimental groups ($p < .001$). This difference was most pronounced on days 16, 17, and 18 — the 3 days immediately after exposure. By day 22, the POP value for groups B and C had resumed their baseline levels.

Passive Closing Pressure. There were slight variations in pressure levels on individual days between the control group and both experimental groups (Fig 4B). However, no significant differences existed between group A and either group B or group C.

Additionally, although there were some variations in mean PCP levels between the experimental groups (Fig 4B), most notably on day 18, no overall significant difference existed between group B and group C.

Active Clearance of Positive Pressure. The control group repeatedly exposed to PBS showed no variation in ACPP throughout the test period. The rats in both experimental groups exhibited an inability to actively clear positive pressure from the ME as compared to controls (Fig 4C). The overall increase was significant for group B ($p < .001$) and group C ($p < .001$) as compared to group A.

The ACPP values generally increased with time. In group B, the ACPP was elevated significantly over baseline and control levels on day 16 (after multiple exposures), day 17, and day 18. On day 22, the ACPP values dropped. Although day 22 showed improved ET function, the ACPP levels remained significantly elevated over the control and baseline measurements. Group C showed similar results. The ACPP levels increased in a stepwise fashion from day 16 through day 18, but dropped on day 22. In group C, all pressure values were significantly elevated over control and baseline measurements ($p < .001$ in both cases). A comparison between the mean pressure values for groups B and C identified no significant difference.

Active Clearance of Negative Pressure. The control rats showed no changes in pressure values from baseline throughout the test period. The rats in both experimental groups exhibited an inability to clear negative pressure from the ME as compared to controls (Fig 4D). The overall difference was significant for group B ($p < .001$) and group C ($p < .001$) versus group A.

Time-dependent changes for ACNP were similar to those for ACPP. In group B, the ACNP values increased significantly over baseline and control values on days 16, 17, and 18. The values dropped on day 22. Day 22 showed improved ET function, but the ACNP levels remained significantly elevated over control and baseline measurements. In group C, negative pressure levels increased in a stepwise fashion from day 16 through day 18, but dropped on day 22. All pressure values were significantly elevated over control values and baseline measurements ($p < .001$ in both cases).

When comparing groups B and C to one another, we observed no overall significant differences. On individual days, however, some differences were found. There was a significant difference between the experimental groups on day 22; the group B values were elevated over the group C values ($p = .002$).

DISCUSSION

The results of the present study demonstrate that repeated transtympanic exposure of the rat ME to simulated GER causes transient abnormal ET function. When exposed to transtympanic pepsin-HCl, experimental rats displayed an inability to appropriately clear applied positive or negative pressure from the ME. This impaired clearance function was dependent on the time since the last exposure, with the greatest impairment occurring 3 days after the last exposure. There was, however, improvement in function by the seventh day after the final exposure. The concentration of pepsin in the HCl had little effect on the active clearance of pressure from the ME. In contrast, ME exposure to pepsin-HCl caused inconsistent changes in the POP. Rats given the lower concentration of pepsin-HCl had elevated POP values, whereas those given the higher concentration had depressed POP values. The PCP values were not significantly affected in either experimental group.

Experiments. Abundant literature exists on the topic of pediatric GER, but it focuses primarily on causes, pathogenesis, and treatments for the disease. The purpose of this experiment was to look at potential sequelae of GER disease, specifically OME. As previously noted, research in this area is limited, and only Butehorn et al[10] have attempted to develop a rat model relating GER and ETD. These researchers showed that a single ME exposure to 0.5 mg of pepsin in 1 mL of 0.01N HCl caused elevated POPs of the ET. The POPs increased on days 1 and 2, with peak values achieved on day 3; the POPs returned to baseline by day 7. The present experiment is different in 3 important ways. First, active ET function tests were used along with the passive function tests for a more accurate measure of ETD. Second, a more accurate model of GER was used for applying the test solutions: rats were exposed multiple times over several days. Third, a second, stronger concentration of pepsin in HCl was used — one that parallels the concentration of crude gastric juice.[13]

Given our experimental results, we believe some tests need more discussion. The POP test or variations on this test have been used for some time to evaluate ET function in normal and diseased states. Fornadley and Burns[17] demonstrated elevations in ET opening pressures in gerbils with OME. Our study found an elevated POP in rats repeatedly exposed to pepsin-HCl, but surprisingly, this finding was not present in both concentration groups. The rats in group C, those exposed to 2.0 mg of pepsin per milliliter of HCl, had significantly lower POPs than did the controls.

The differences exhibited by the rats in these two concentration groups are not easily explained, and these results alone are not sufficient to draw conclusions. Our ongoing research includes functional and morphological evaluation of ET mucosa using these different concentrations of pepsin in HCl. These future data may give us some insight into the variable effect of pepsin on POP.

The active function tests used in this protocol provide a necessary link between ETD and the potential effects of GER. It has been accepted for some time that the most important function of the ET is pressure equilibration between the ME and the nasopharynx.[7] A breakdown in this function is involved in the pathogenesis of OME. In rats repeatedly exposed to pepsin-HCl solution, there was a significant inability to clear positive pressure from the ME. Positive pressure is uncommon in the ME, except in certain situations like the Valsalva maneuver or ascent in an airplane. The relevance of this test is that it demonstrates a critical deficiency in normal ET function after repeated pepsin-HCl exposure.

An inability to equilibrate ME negative pressure is regarded as the primary deficit in ETD. There is documented evidence that children with ME effusions spend more time with negative ME pressures than those without ME effusions.[18] Additionally, animal studies have demonstrated a fundamental link among experimentally induced ETD, development of ME negative pressures, and occurrence of OME.[19] The fact that simulated GER led to impaired clearance of negative pressure suggests that GER may be related to the pathogenesis of ETD.

Moreover, of the 3 tests demonstrating significant changes from the control state, all showed a similar time-related pattern. Pressure changes occurred over the 3 days after the most recent exposure, reaching a maximum by day 3, but tending toward baseline on day 7. These results were not unexpected. Butehorn et al[10] noted that maximal passive pressure test changes were observed on day 3 after the experiment began. These researchers followed pressures every other day from day 3 through 9 and determined that after day 3 there was a rapid return toward baseline values. The baseline values were reached by day 7, and there were no further changes on day 9. Because of these results, the pressures in our experiment were measured on days 1, 2, 3, and 7 after the final exposure. The trend toward baseline on day 7 may represent partial recovery or healing. However, some impairment still exists, as the ACPP and ACNP remain significantly elevated at 7 days.

Study Limitations. In any experiment, there are factors that may limit the overall significance of the findings. For example, the design of the current experiment is deficient in that rats were exposed transtympanically to the test solution: pepsin-HCl (or PBS) was placed directly into the ME so that it passed over the entire course of the ET mucosa. This method could have artificially enhanced the experimental results, because the entire mucosal surface of the ET was exposed, and exposure time was prolonged. It is our hope that future models will circumvent this problem. Second, the animals used in this study displayed a broad range of weights (240 to 410 g). However, although it may be true that weight and age can affect baseline ET function, White et al[20] found that there is no significant relationship between age or weight and baseline ET function. Our rats showed no significant difference in baseline ET function between the control and experimental groups.

Ongoing Research. Our existing model was improved over the early design used by Butehorn et al.[10] The present experiments use a rat model in which gastric juice components are placed directly into the nasopharynx via a surgically implanted catheter. This experimental design is intended to simulate true GER as it moves into the nasopharynx. The rats are conscious and able to swallow throughout the trials.

CONCLUSION

Gastroesophageal reflux can be linked to many pediatric medical disorders. Our experiments demonstrated that simulated GER leads to ETD in rats. Given these results, one must consider that GER may lead to ETD and subsequent OME in humans. In evaluating the patient with chronic OME, the history might include questions regarding the presence of GER, persistent regurgitation, recurrent cough, asthma, chronic sinusitis, and glottic and/or subglottic abnormalities. When the findings are positive, further studies including endoscopic examination and dual-probe pH monitoring may be warranted.

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PHONATORY MECHANISMS AFTER VERTICAL PARTIAL LARYNGECTOMY WITH GLOTTIC RECONSTRUCTION BY FALSE VOCAL FOLD FLAP

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A prospective randomized study was designed to compare phonatory mechanisms in patients treated with frontolateral partial laryngectomy (FLPL) with or without glottic reconstruction with false vocal fold (FVF) mucosal flap. Objective voice assessment was based on computerized acoustic recordings and videolaryngostroboscopy performed before, 1 year after, and 2 years after surgery. Phonatory mechanisms were studied according to the surgical technique and correlated with acoustic recordings. The results showed that supraglottic structures participated in laryngeal closure and voice production in 80% of cases in which a standard FLPL was performed. The FVF mucosal flap was involved in both laryngeal closure and voice production in 54% of patients treated with glottic reconstruction. The type of laryngeal closure (glottic or supraglottic) statistically influenced the acoustic measurements. As glottic closure is more frequent in patients treated with glottic reconstruction, the authors recommend performing reconstruction of the glottis with an FVF mucosal flap when an FLPL is indicated.

KEY WORDS — acoustic measurements, glottic reconstruction, laryngeal closure, vertical partial laryngectomy, videolaryngostroboscopy.

INTRODUCTION

Vertical partial laryngectomies (VPLs) include open laryngeal procedures: laryngofissure with cordectomy, frontolateral partial laryngectomy (FLPL), the anterior commissure procedure, and hemilaryngectomy. These techniques resect the true vocal fold and a wide part of the paraglottic space, drastically modifying laryngeal closure. Glottic insufficiency is a common finding after VPL, with potential aspiration and vocal dysfunction. Inadequate subglottic pressure and air leak during phonation explain the weak and breathy voice associated with glottic incompetence.

In order to reduce the vocal effects of surgery, glottic reconstruction is performed to restore the competence of the glottis after VPL by an external approach.[1] This goal is achieved when complete laryngeal closure is obtained during normal phonation, allowing adequate subglottic pressure for voicing. Additionally, the remaining true vocal fold should contact the opposite, reconstructed side during phonation to provide better vocal results.[2]

Despite the lack of standard methods for voice evaluation, videolaryngostroboscopy is widely used in clinical practice.[3,4] It is of particular interest after VPL with glottic reconstruction for studying laryngeal closure during phonation and for evaluating the functional outcome of the tissue used for glottic reconstruction.

We previously found that glottic reconstruction with a false vocal fold (FVF) mucosal flap[5] improved acoustic measurements after FLPL.[6] This report is a follow-up of this earlier prospective study. Videolaryngostroboscopy was performed on the same patients and correlated with acoustic recordings to identify phonatory mechanisms after FLPL with or without glottic reconstruction. The aim of this study was not to discuss the oncological indications in the treatment of glottic carcinoma, but rather to study mechanisms of phonation after external surgical resection with or without glottic reconstruction.

PATIENTS AND METHODS

This prospective clinical study was conducted in 27 patients treated by FLPL for a previously untreated primary T1aN0 squamous cell carcinoma of the glottis. The mean age of the patients was 65.3 years (range, 35 to 83 years). None of the patients were voice professionals. The surgical procedure and acoustic recording methodology have been previously reported.[5-7] The FLPL was performed by an external midline thyrotomy and resected in all cases a portion of the thyroid cartilage, the true vocal fold with the paraglottic space on the tumor-bearing side, the

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STROBOSCOPIC DATA

	At 1 Year			*At 2 Years*		
	+GR (n = 13)	*−GR (n = 10)*	*Total (n = 23)*	*+GR (n = 13)*	*−GR (n = 7)*	*Total (n = 20)*
Arytenoid mobility						
Normal	11	6	17	11	4	15
Diminished	2	4	6	2	3	5
Neoglottic closure						
Complete	7 (54%)	1 (10%)	8 (34%)	7 (54%)	1 (14%)	8 (40%)
Incomplete	2 (15%)	1 (10%)	3 (13%)	2 (15%)	1 (14%)	3 (15%)
Supraglottic	4 (31%)	8 (80%)	12 (53%)	4 (31%)	5 (72%)	9 (45%)

GR — glottic reconstruction.

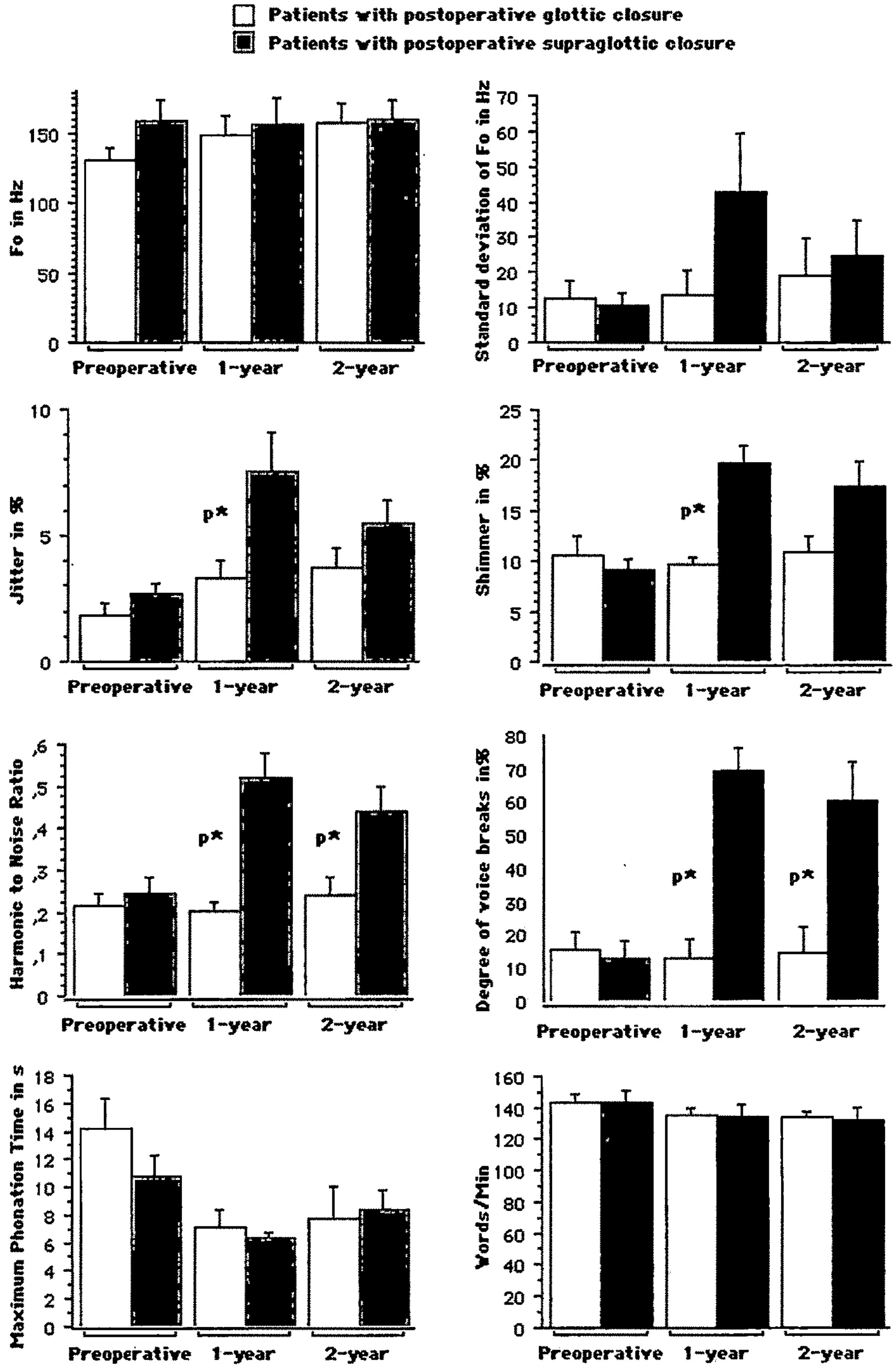
anterior commissure, the floor of the ventricle, and the vocal process of the arytenoid cartilage. The cricoid cartilage and the FVF were never resected. For glottic reconstruction, the FVF was carefully dissected from the remaining thyroid cartilage. The inferior edge of the mucosal flap was then pulled down and sutured to the inferior margin of the resection. A double-blind randomization for glottic reconstruction was performed before surgery. Fourteen patients were included in the arm with glottic reconstruction, and 13 patients in the arm with standard FLPL. Preoperative and postoperative vocal analyses, including acoustic and videolaryngostroboscopic recordings, were available in 23 patients at 1 year after surgery (13 patients with glottic reconstruction and 10 with standard FLPL). At 2 years after surgery, 20 had both acoustic and videolaryngostroboscopic recordings (13 with glottic reconstruction and 7 with standard FLPL). All patients had postoperative speech therapy.

Speech and acoustic voice evaluation was performed with the Computerized Speech Lab and the Multidimensional Voice Program (Kay Elemetrics, Pine Brook, NJ). The frequency measurements under analysis were the average fundamental frequency (F0) in hertz, the F0 standard deviation (F0 SD) in hertz, the jitter (period-to-period variation of F0 or pitch pertubation) in percent, the shimmer (period-to-period variation of voice amplitude or amplitude pertubation) in percent, the harmonics-to-noise ratio (ratio of the energy of the harmonic components to that of unharmonic or noise components), and the degree of voice breaks (number of times F0 becomes undetectable or "voiceless") in percent. A sustained vowel /a/ was examined at the patient's habitual pitch and loudness. Durational measurements included the maximum phonation time in seconds and the speech rate, obtained by reading a phonetically balanced French text (*Grand Mere raconte*),[7] in words per minute.

Laryngostroboscopic evaluation during phonation of a sustained vowel /i/ was performed with a 0° rigid endoscope (Wolf-Atmos Medical France, Marseille, France) connected to a halogen light source. For videolaryngostroboscopy, a charge-coupled video camera (Panasonic KS 152-Pouret, Paris, France) was connected to the endoscope, and images were recorded on a Sony ³/4-inch videotape recorder (VO-5800 PS model, Solal, Strasbourg, France). All of the videostroboscopic recordings were reviewed by a trained speech pathologist and a laryngologist. Evaluation of the neoglottis after FLPL was based on the following parameters.

First, postoperative glottic closure in phonation was rated as complete, incomplete, or supraglottic. Complete glottic closure was achieved in patients whose remaining true vocal fold completely contacted the opposite side. This condition was obtained in the reconstructed larynx if the true vocal fold contacted the FVF mucosal flap. Patients with a longitudinal glottal gap during phonation had incomplete glottic closure. When a high degree of supraglottic dysfunction obstructed visualization of the true vocal folds, the laryngeal closure was rated as supraglottic. We distinguished supraglottic closure with the FVFs moving to the midline from supraglottic closure by partial or complete anteroposterior constriction of the larynx with the arytenoid cartilages moving toward the epiglottis.[8]

Second, the postoperative arytenoid mobility was rated as normal or impaired. To study the influence of neoglottic characteristics on voice quality after FLPL, we performed a statistical analysis between the frequency and durational measurements obtained by computerized recordings and the anatomic parameters of arytenoid mobility and glottic closure. A repeated analysis of variance (ANOVA) and an unpaired t-test were used in each case to compare the preoperative and postoperative evolution of the acoustic features between groups. Additionally, a χ^2 test was performed to analyze the relationship between glottic reconstruction and the previously listed parameters. In all cases, the statistically significant level was fixed at .05.



Acoustic data (mean ± SE). p* — significant difference ($p < .05$).

RESULTS

PREOPERATIVE VIDEOLARYNGOSTROBOSCOPIC RECORDINGS

In all patients except 1, the preoperative glottic closure was rated as complete. One patient, with a bulky pedicled tumor, had incomplete glottic closure. Mucosal waves were always absent on the tumor-bearing fold and present on the opposite fold. In 2 cases, mucosal waves in phonation were noted on the FVF that partially covered the tumor-bearing true vocal fold. Even though a slight degree of supraglottic dysfunction was noted in these 2 cases, no patient in the study had preoperative supraglottic closure.

POSTOPERATIVE VIDEOLARYNGOSTROBOSCOPIC RECORDINGS

Three patients, 2 of whom had glottic reconstruction, had temporary grade 1 aspiration.[9] However, at 1 postoperative year, no patients had aspiration.

Neoglottic Closure in Phonation. Twenty-three patients were available for videolaryngostroboscopic evaluation of glottic closure at 1 year. The results are summarized in the Table. Supraglottic closure was observed in 53% of cases, whereas complete glottic closure was present in 34% of cases. Three patients (13%) had incomplete glottic closure and were excluded from the acoustic data analysis. The acoustic data of the 20 remaining patients with postoperative complete closure or supraglottic closure are summarized in the Figure. As tested with an ANOVA, jitter, shimmer, the harmonics-to-noise ratio, and the degree of voice breaks were significantly different between patients with complete glottic closure and patients with supraglottic closure. The mean values in patients with complete glottic closure and supraglottic closure were 3.3% and 7.5%, respectively, for jitter (p = .05); 9.6% and 19.6%, respectively, for shimmer (p = .02); 20% and 52%, respectively, for the harmonics-to-noise ratio (p = .002); and 13.0% and 69.3%, respectively, for the degree of voice breaks (p = .0007). The χ^2 test showed a significant relationship between complete glottic closure and glottic reconstruction. Eighty-seven percent of the patients with complete glottic closure had undergone glottic reconstruction, whereas 66% of the patients with supraglottic closure had undergone a standard FLPL (p = .02).

Two-year postoperative recordings were collected in 20 patients and are summarized in the Table. There was no difference in laryngeal closure status between the 1-year and 2-year recordings for the individual patients. Complete glottic closure was observed in 8 patients, and supraglottic closure in 9 patients. The 2-year acoustic data from patients with complete glottic closure and supraglottic closure are summarized in the Figure. The mean values in patients with complete glottic closure and supraglottic closure were 24% and 44%, respectively, for the harmonics-to-noise ratio (p = .05) and 14% and 60%, respectively, for the degree of voice breaks (p = .05). The other acoustic measures were not significantly different between the two groups.

At 2 years, 87% of the patients with complete glottic closure had undergone glottic reconstruction, whereas 55% of the patients with supraglottic closure had undergone a standard FLPL. This difference was not significant (p = .17). However, it was of note that 3 patients with supraglottic closure 1 year after surgery were lost to follow-up between 1 and 2 years.

Arytenoid Mobility. The 1-year postoperative arytenoid mobility was rated as impaired in 6 patients (see Table). The acoustic measurements were not significantly different according to whether patients had normal or impaired arytenoid mobility, as tested with an ANOVA (data not shown).

Glottic reconstruction was not statistically related to arytenoid mobility (p = .23). On the other hand, arytenoid mobility statistically influenced postoperative glottic closure. The 6 patients with impaired arytenoid mobility had supraglottic closure (p = .04).

Two years after surgery, 5 of the 20 patients available for the study had impaired arytenoid mobility (see Table). These patients all had supraglottic closure (p = .01).

DISCUSSION

Phonation involving supraglottic structures is a poorly understood voice disorder in which supraglottic structures vibrate in place of the true vocal folds. Adduction of the FVFs to the midline, also called ventricular dysphonia, is the most commonly observed finding. Additionally, sphincterlike closure of the larynx can be noted, with an anteroposterior contraction of the glottis. Van Doersten et al[10] suggested that this voice disorder was basically a compensatory mechanism for glottic dysfunction. Various studies support this hypothesis, demonstrating resolution of ventricular dysphonia after treatment of the primary vocal disease.[10-14]

Previously published results have shown that supraglottic structures frequently participate in phonation after VPL. Blaugrund et al[15] reported on 20 patients treated with various VPL techniques and noted with laryngostroboscopy that supraglottic structures were involved in phonation in 80% of cases. Similar results were found after the open laryngeal technique[2] and after endoscopic laser resection for glottic carcinoma.[16] Authors have concluded that supraglottic contraction in phonation was a compensatory mechanism for postoperative glottic incompetence.[2,15,16] Therefore, when a glottic reconstruction is performed to improve vocal function, laryngeal closure evaluation is an important issue in vocal assessment. A glottic reconstruction technique that allows the remaining true vocal cord to contact the opposite, reconstructed side during phonation might be associated with a better vocal result. Likewise, persistent glottic incompetence after reconstruction might be associated with a glottal gap in phonation and might lead to supraglottic compensation.

In our study, the laryngeal sphincter was effective in all cases in preventing clinical aspiration 1 year after surgery. In most cases, laryngeal closure was complete; only 3 patients had a persistent glottal gap in phonation. However, the structures participating in laryngeal closure were significantly different in patients treated with FLPL according to whether they had an FVF mucosal flap. Patients with standard FLPL had supraglottic closure during phonation in 80% of cases. On the other hand, patients with glottic reconstruction had complete glottic closure at 1 year in 54% of cases, versus 10% in patients without glottic reconstruction. Only 2 cases showed a persistent glottal gap and were considered vocal failures. Because our results were not statistically significant at 2 years, further studies are being conducted on a larger number of patients.

The FLPL technique resects the vocal process of the arytenoid cartilage. For oncological reasons, when the tumor is close to the vocal process, a partial resection of the arytenoid body is performed, with potential postoperative impairment of arytenoid mobility. In our study, arytenoid mobility was not statistically related to glottic reconstruction. However, the patients with impaired arytenoid mobility all had postoperative supraglottic closure. This phonatory behavior had already been reported in patients with unilateral vocal fold paralysis.[10] Our results after FLPL suggested that impaired arytenoid mobility might lead to poor glottic closure and a tendency toward supraglottic compensation.

Acoustic analysis demonstrated that the type of laryngeal closure influenced the final vocal results. Patients with glottic closure had better 1-year frequency measures than patients with supraglottic closure. Jitter and shimmer were higher in patients with supraglottic closure. The poor vibrational properties of the FVFs, as compared with those of the true vocal folds, could participate in the worse harmonics-to-noise ratio achieved in patients with supraglottic compensation. Even though 3 patients were lost to follow-up at 2 years after surgery, the differences persisted between patients with glottic versus supraglottic laryngeal closure.

The physiopathology of supraglottic contraction in phonation remains unknown. Histologic preparations have found various degrees of FVF hypertrophy composed of glandular and fibrous connective tissue without muscular hypertrophy.[10,17] These findings supported surgical excision of the FVF after speech therapy failure[18] in patients with adduction of the FVFs to the midline. Kendall and Leonard[11] reported resolution of ventricular dysphonia after supraglottic Botox injection in 7 patients affected by various laryngeal diseases. They suggested that muscular structures, such as the thyroarytenoid muscle or the lateral cricoarytenoid muscle, were involved in supraglottic phonation. As the thyroarytenoid muscle is widely resected at the time of FLPL, other intrinsic laryngeal muscles might be responsible for the postoperative supraglottic closure. Further experimental studies are needed to elucidate the underlying mechanisms of supraglottic contraction during phonation after FLPL.

In conclusion, our results showed that postoperative arytenoid mobility and glottic reconstruction by FVF flap were factors that influenced laryngeal closure after VPL. Supraglottic compensation occurred more frequently in patients treated without glottic reconstruction. The FVF mucosal flap participated in phonation without supraglottic compensation in the reconstructed larynx. As laryngeal closure influenced objective acoustic measurements in this study, we suggest performing an FVF mucosal flap reconstruction of the glottis when an FLPL is indicated.

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ADDUCTOR SPASMODIC DYSPHONIA AND BOTULINUM TOXIN TREATMENT: THE EFFECT ON WELL-BEING

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Adductor spasmodic dysphonia (AdSD) is a controversial and enigmatic voice disorder. It is generally accepted that it has a neurologic, although undetermined, cause, and it is accompanied by much psychological and physical distress. In this prospective study, standardized psychometric tests were used to assess the personality characteristics and psychological and somatic well-being of 46 patients with AdSD. Moreover, the effect of botulinum toxin (Botox) treatment on their well-being was evaluated. No significant differences could be detected between patients and a representative norm group concerning 7 personality characteristics. Nevertheless, before treatment, there were significantly more psychological and somatic complaints. After establishment of a normal to near-normal voice with Botox injections, these complaints were reduced to normal levels — a finding suggesting these phenomena to be secondary to the voice disorder. These findings, and the normal personality characteristics, do not support a psychogenic cause of AdSD.

KEY WORDS — adductor spasmodic dysphonia, botulinum toxin, psychometry.

INTRODUCTION

Adductor spasmodic dysphonia (AdSD) is an uncommon and poorly understood voice disorder that was first described by Traube[1] in 1871. The vocal symptoms are characterized by difficulty with voice initiation, a strain-strangled, effortful phonation with voice breaks, and glottal fry. Remarkably, these symptoms are reduced or absent during whispering, speaking or singing in a falsetto register, and nonspeech vocalizations (laughing, yawning). Spasmodic dysphonia is initially intermittent and task- or situation-specific. The marked intermittency and functional specificity of the symptoms have suggested a psychological basis for this unusual disorder.

Initially, (adductor) spasmodic dysphonia was considered predominantly as a conversion symptom of a hysterical illness affecting the coordination of speaking and breathing.[2-4] Heaver[5] argued that spasmodic dysphonia especially affected persons with a hysterical and narcissistic personality structure. Berendes[6] analyzed several psychological factors in 23 patients with spasmodic dysphonia. He concluded that this voice disorder had to be considered as a neurosis without hysterical characteristics. However, these studies could offer no objective support for the hypothesized contribution of psychological factors in the ontogeny of spasmodic dysphonia, because they were based on case histories or patient descriptions.

To bypass the questionable reliability of interviewers and observers, psychometric tests were applied to assess the psychological phenomena related to this enigmatic voice disorder.[7-10] These studies showed elevated levels of depression and anxiety and negative attitudes toward communication in AdSD patients as compared to matched normal controls. In one study, elevated levels of somatic complaints were also found.[8] Treatment with botulinum toxin (Botox) injections generally reduced the levels of depression and anxiety, and significant attitudinal improvement was observed. Whether these differences could be explained as a reaction to a chronic disorder or as being related to a possible psychogenic origin of spasmodic dysphonia remained unsolved.

Nowadays, spasmodic dysphonia is thought to be a physical disorder, an idea already proposed by Schnitzler[11] in 1874. This is supported by the association of spasmodic dysphonia with other focal and generalized dystonias or involuntary movements, such as myoclonus and tremor. It is now assumed that AdSD has a neurologic (undetermined) cause, and it is characterized as a disorder of central motor

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TABLE 1. DEMOGRAPHIC DATA

		Age (y)		Duration of Symptoms (y)	
	No.	Mean	Range	Mean	Range
Women	32	47.9	19-80	8.9	1-29
Men	14	53.7	31-68	7.9	1-31

processing resulting in focal laryngeal dystonia. As well as the abnormal voice, a variety of other abnormalities have been found on objective tests, including electroencephalography, evoked potentials, blink reflexes, vocal reaction times, and responses to vagal stimuli.[12]

In summary, AdSD is generally accepted as a physical voice disorder associated with several psychological and somatic complaints. Some studies on the psychological impact of AdSD have been performed. The results of these studies supported the need for further research into the role of emotional aspects of spasmodic dysphonia prior to intervention, their relationship to objective and subjective voice changes, and how therapeutic management affects the long-term outcome of patients with spasmodic dysphonia.[10] At present, the role of general personality variables in relation to AdSD is unknown.

The aim of this prospective study was twofold: 1) to investigate the hypothesis that personality variables might underlie spasmodic dysphonia and 2) to determine whether symptoms of negative affectivity (depression and anxiety) and somatic complaints are consequences of the disease.

SUBJECTS AND METHODS

Forty-six consecutive patients (32 female and 14 male) with AdSD were included in this study. The demographic data are summarized in Table 1. On the basis of their medical history, none of the patients appeared to have psychiatric or neurologic disorders, particularly, no other dystonias or familial tremors. All patients had had symptoms of AdSD for more than 1 year and had no previous treatment with Botox.

The personality characteristics of the patients were assessed with the Dutch Personality Questionnaire (DPQ).[13] This questionnaire was developed in the early 1970s in the Netherlands and was adapted from the California Psychological Inventory.[14] Extensive validational data are available on this inventory. The reliability and validity of the DPQ have been proven to be satisfactory.[13,15-19] The 132 items of the DPQ, answered on a 3-point scale (True, ?, and False), are non-overlappingly keyed in the following scales: 1) neuroticism (21 items); 2) social anxiety (15 items); 3) rigidity (25 items); 4) hostility (19 items); 5) egotism (16 items); 6) dominance (17 items); and 7) self-esteem (19 items). The 7 scales are not independent: the highest scale intercorrelations are approximately .45. Usually, 3 second-order factors are found: emotional stability versus neuroticism (high loadings of scale 7 and with opposite sign scale 1); introversion versus extraversion (high loadings of scale 2 and with opposite sign scale 6); and dogmatism versus friendliness (high loadings of scales 3, 4, and 5).[20] The raw scores on the 7 DPQ scales were used in this study.

The Hopkins Symptom Checklist[21] (HSCL) was applied to assess and monitor the psychological and somatic complaints existing before and during Botox treatment. This checklist is a self-report symptom rating scale that a subject answers on a 4-point scale of distress ("not at all" to "extreme"). In answering the HSCL, an explicit temporal referent is provided in terms of "How have you felt during the past seven days including today?" This makes the HSCL suitable for measuring changes, eg, measuring the effect of therapy. The Dutch version of the HSCL consists of 57 symptoms and complaints and has the following scales: somatic well-being (HSCL-Somat, 8 items), psychological well-being (HSCL-Psych, 17 items), and total score (HSCL-Total, 57 items). In this study, the norm group of the general population was used as a reference. There is ample support for the reliability and validity of the HSCL.[21]

To substantiate the effect of Botox treatment on the changes in psychological and somatic complaints, we also determined the changes in vocal function through subjective (patient) and objective (observers) ratings. The subjective rating consisted of statements concerning the patient's experience of his or her voice quality and performance (intelligibility, effort, and fluency of speech). Perceptual evaluation of spontaneous speech was used to rate voice quality objectively. All voice samples were judged by 3 experienced observers who were familiar with both spasmodic dysphonia and a perceptual evaluation rating system derived from the GRBAS system.[22] For the purposes of this study, we only used the "G" parameter (grade) to quantify the overall impression of severity of the dysphonia. Assessment of the degree of dysphonia was achieved by calculating the means of the scores of the 3 observers. Both subjective and objective ratings were performed on visual analog scales. On the score form, each parameter was scored on a continuous horizontal line of 10 cm from bad or extremely pathological on the left side to good or normal on the right side. The distance in centimeters from the left side measured the score.

This study was part of a larger prospective clinical trial in which different procedures of Botox injections were compared in AdSD patients.[23] All 46 patients

TABLE 2. SCORES ON DUTCH PERSONALITY QUESTIONNAIRE[13]

	Spasmodic Dysphonia Group (n = 46)	*Dutch Norm Group (n = 5,686)*
Neuroticism	13.2 ± 9.4	12.0 ± 7.9
Social anxiety	10.2 ± 8.7	10.9 ± 6.9
Rigidity	26.9 ± 9.9	28.4 ± 7.8
Hostility	16.5 ± 7.8	18.2 ± 6.7
Egotism	11.2 ± 5.0	12.6 ± 5.0
Dominance	14.6 ± 6.7	13.8 ± 5.8
Self-esteem	27.8 ± 6.3	28.0 ± 5.6

Data are mean ± SD. None of scales differed significantly; 1-sample *t*-test, *df* = 45.

TABLE 3. PRETREATMENT SCORES ON HSCL[21] AND NORMATIVE DATA

	Spasmodic Dysphonia Group (n = 46)	*Dutch Norm Group (n = 406)*	*p**
HSCL-Psych	12.6 ± 8.6	7.9 ± 6.2	.0005
HSCL-Somat	3.5 ± 2.6	2.1 ± 2.3	.0005
HSCL-Total	36.8 ± 20.0	22.3 ± 16.5	<.0001

HSCL — Hopkins Symptom Checklist, Psych — psychological well-being, Somat — somatic well-being.

Data are mean ± SD.

*One-sample *t*-test, *df* = 45.

underwent unilateral and bilateral injections. During the first procedure, 5 units (1.25 U/0.1 mL) of Botox were injected in the left thyroarytenoid muscle only. The second treatment was performed after the voice quality had returned to the preinjection level (established by the patient and by audio and video registrations). During this procedure, both thyroarytenoid muscles were injected, each with 2.5 units (1.25 U/ 0.1 mL) of Botox. Under electromyographic guidance, a monopolar 27-gauge 30-mm Teflon-coated needle was inserted percutaneously, through the cricothyroid membrane and into the left thyroarytenoid muscle during the first procedure, and into both thyroarytenoid muscles during the second procedure.

The choice for the next treatment depended on the patient's preference. The dose was adjusted by titrating efficacy and side effects. The end of the study was the moment at which the patient experienced his or her former "normal" voice. However, a normal voice could not always be established. In those cases, the end point was the optimal voice that could be achieved.

The questionnaires were administered at the following times. The moment the patients entered the study (pretreatment), they were asked to complete the DPQ, the HSCL, and the self-rating questionnaire for (subjective) judgment of the voice. At that time, recordings were made for perceptual (objective) evaluation of the voice quality. During the follow-up period, ie, 1 month after the unilateral injection, 1 month after the bilateral injection, and at the moment of the optimal voice (posttreatment), all tests except for the DPQ were performed again.

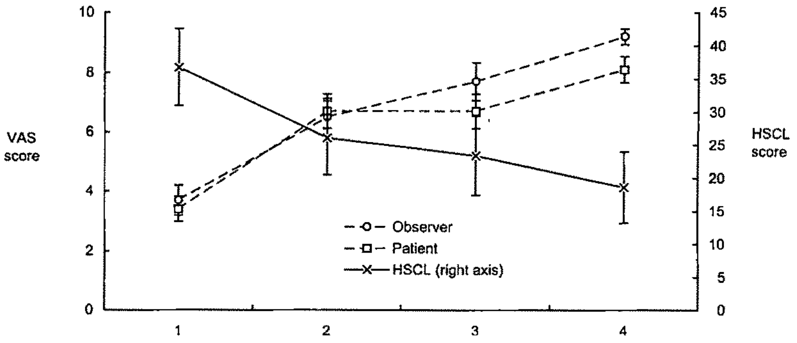
RESULTS

Table 2[13] provides descriptive statistics for the DPQ scores of the 46 AdSD patients prior to treatment with Botox. There were no significant differences between the patients and the representative norm group of the Dutch population in the raw scores of the 7 scales of the DPQ. On the other hand, the HSCL showed differences across all 3 scales, patients having significantly elevated mean scores compared to the representative norm group (Table 3[21]).

The Figure[21] shows the changes in the patients' and observers' voice ratings and HSCL-Total scores over the course of time. Both the visual analog scale scores of vocal function and the HSCL scores showed continuous improvement during Botox treatment. These findings are reflected in Table 4,[21] as well. Compared to the mean pretreatment scores of the HSCL, there was a significant decrease after treatment. In fact, the mean scores of all 3 scales reached values within the normal range. Comparing the scores of the AdSD patients after treatment with the representative norm group, we found no significant differences (Table 5[21]).

DISCUSSION

The first goal of this study was to investigate the hypothesis that personality variables might underlie



Changes in patients' and observers' voice ratings (visual analog scale [VAS] scores) and Hopkins Symptom Checklist (HSCL)–Total[21] scores over course of time. Means are given, with associated 95% confidence intervals. Changes over time were statistically significant ($p < .001$) according to mixed-model analysis of variance. 1 — moment before Botox injection; 2 — 1 month after unilateral Botox injection; 3 — 1 month after bilateral Botox injections; 4 — moment of "best voice ever since."

TABLE 4. PRETREATMENT AND POSTTREATMENT SCORES ON HSCL[21] AND RATINGS OF VOICE QUALITY

	Pretreatment	*Posttreatment*	*p**
Patient rating of voice quality†	3.4 ± 1.4	8.1 ± 1.5	<.001
Observer rating of voice quality†	3.7 ± 1.7	9.2 ± 0.9	<.001
HSCL-Psych	12.6 ± 8.6	6.1 ± 6.4	<.001
HSCL-Somat	3.5 ± 2.6	2.2 ± 2.6	.005
HSCL-Total	36.8 ± 20.0	18.6 ± 18.4	<.001

Data are mean ± SD.

*Paired *t*-test.

†Zero represents "very bad or extremely pathological" voice, and 10 represents "good or normal" voice.

AdSD. Using the DPQ, we found no significant differences between the AdSD patients and the representative norm group of the Dutch population. On the basis of the DPQ results, no distinction could be made between normal and AdSD subjects. Thus, either there are no typical personality traits associated with AdSD or the DPQ could not detect those personality attributes. As the DPQ is accepted as an accurate, comprehensive tool, it would seem that the first hypothesis (AdSD is not associated with typical personality traits) is justifiable.

Our findings parallel the early work of Aronson et al,[7] who applied the Minnesota Multiphasic Personality Inventory (MMPI) to 33 patients with spasmodic dysphonia. None of the 10 clinical scales of the MMPI could differentiate between the spasmodic dysphonia patients and a general medical outpatient population. On the basis of the MMPI, the authors argued that patients with spasmodic dysphonia had to be a remarkably heterogeneous group.

Our second aim was to determine whether symptoms of negative affectivity (depression and anxiety) and somatic complaints were consequences of the disease. Research on patients with physical symptoms or disorders underlines the changeability of psychological and somatic complaints in relation to medical treatment.[24] In 1991, Cannito[8] found elevated scores of depression and anxiety among 18 patients with spasmodic dysphonia compared to matched normal controls. In addition, the patients showed abnormally elevated levels of somatic complaints. Whether these differences could be explained on the basis of a reaction to a chronic disorder or were related to a possible psychogenic origin of spasmodic dysphonia remained unsolved.

Before intervention, we also found statistically significant elevated levels of psychological and somatic complaints. These complaints decreased systematically during the course of the treatment. Compared to the pretreatment scores of the HSCL, a significant reduction was found after treatment (Table 5). Moreover, the scores all reached levels within the normal range. The subjective and objective ratings of voice quality also improved systematically during treatment, establishing a normal to near-normal voice. These findings suggest that the higher pretreatment scores of psychological and somatic complaints were secondary to the voice disorder.

TABLE 5. POSTTREATMENT SCORES ON HSCL[21] AND NORMATIVE DATA

	Spasmodic Dysphonia Group (n = 46)	*Dutch Norm Group (n = 406)*	*p**
HSCL-Psych	6.1 ± 6.4	7.9 ± 6.2	.06
HSCL-Somat	2.2 ± 2.6	2.1 ± 2.3	.60
HSCL-Total	18.6 ± 18.4	22.3 ± 16.5	.18

Data are mean ± SD.

*None of scales differed significantly; 1-sample *t*-test, *df* = 45.

In just 2 studies has the effect of treatment of spasmodic dysphonia on standardized psychometric test results been assessed.[9,10] In the first study, Murry et al[9] demonstrated that 32 subjects displayed significantly elevated depression and anxiety in the absence of significantly elevated somatic complaints. The use of Botox had generally reduced the measures of depression and anxiety at 1 week after injection, and the effect was maintained during the ensuing 2 months. The authors concluded that the elevated depression and anxiety could have been the result of acquiring spasmodic dysphonia. These results are in line with ours, but in their study there was a problem in terms of subject compliance. They started with 32 patients, but the long-term influence (defined as 2 months after treatment) could only be determined for 13 of the original 32 subjects, so the study's validity was reduced.

The second study in which patients with spasmodic dysphonia were examined by means of psychometric tests was conducted by the same group.[10] They examined the communication attitudes of 20 patients with AdSD before and after Botox injection. Although significant attitudinal improvement was observed, the patient group remained significantly different in their attitudes toward communication as compared to the normal controls. The amount of change in communication attitudes was significantly related to the amount of change in standardized psychological measures of depression and anxiety. However, this study encountered the same problem as the other study.[9] Initially, 20 patients were included and could be analyzed 1 week after Botox treatment. However, only 14 of the 20 patients returned for the 2-month postinjection follow-up examination. Moreover, before the Botox injection, 6 of the 20 patients' test scores (Erickson Scale of Communication Attitudes) already fell within the normal range.

We have to emphasize that on the basis of the results of our study, no inferences can be made about causality and the role of psychological risk factors in the development of spasmodic dysphonia. However, results of recent research in the area of health psychology demonstrate that personality characteristics are stable constructs that are unrelated to somatic disorders.[25] On the other hand, the response to a somatic treatment does not necessarily rule out a psychogenic contribution to either symptoms or symptom severity. A longitudinal study would be more appropriate for elucidating a possible causal relationship between specific personality attributes and the occurrence of AdSD.

CONCLUSION

In this study, standardized psychometric tests were used to quantify psychological aspects of patients with AdSD. No differences in personality characteristics between AdSD patients and a representative norm group were found. However, the patients showed significantly more psychological and somatic complaints. After establishment of a (near) normal voice with Botox injections, these features were reduced to normal levels. This finding suggests that the psychological and somatic complaints are secondary to the voice disorder. These findings, and the normal personality characteristics, do not support a psychogenic cause of AdSD.

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BIOFILM FORMATION ON VOICE PROSTHESES: IN VITRO INFLUENCE OF PROBIOTICS

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In order to determine the influence of probiotic bacteria on biofilm formation on Groningen and Provox 2 voice prostheses in an artificial throat, we grew biofilms on both types of voice prostheses and exposed them 3 times daily to a probiotic bacterial suspension. As a control, we perfused an artificial throat with phosphate-buffered saline solution. Perfusion with *Lactococcus lactis* 53 suspension reduced the percentage numbers of bacteria and yeasts, respectively, on the Groningen prostheses to 17% and 22% and on the Provox 2 prostheses to 19% and 45%, compared to the number of colony-forming units on the control prosthesis, which was set at 100%. A suspension of *Streptococcus thermophilus* B reduced the percentage numbers of bacteria and yeasts, respectively, on the Groningen prostheses to 53% and 33% and on the Provox 2 prostheses to 14% and 0%, as compared to the control prosthesis. All other probiotic strains tested caused some reduction in the percentages of bacteria or yeasts, but strong differences between the types of prostheses were observed. In conclusion, *L lactis* 53 and *S thermophilus* B strongly reduce the occurrence of yeasts and bacteria in voice prosthetic biofilms.

KEY WORDS — artificial throat, biofilm, laryngectomy, probiotics, voice prosthesis.

INTRODUCTION

Since the introduction of the "duckbill" prosthesis by Blom and Singer in 1979, several types of indwelling silicone rubber voice prostheses have been developed, giving laryngectomized patients the ability to speak again.[1,2] Generally, a combination of the personal preferences of the patient and the physician determines the choice of the type of prosthesis, although most types of prostheses are interchangeable. Colonization of the valve side of prostheses by bacteria and yeasts leads to deterioration of the silicone rubber, leakage of the valve, or a higher airflow resistance, making it more difficult to speak.[3-7] On average, a prosthesis has to be replaced every 3 to 4 months. Replacement presents considerable discomfort for the patient and can be detrimental to the tracheoesophageal fistula, leading to insufficiency, scar tissue formation, or stenosis. Frequent replacements also lead to higher costs for medical care, new prostheses, and travel expenses for the laryngectomized patients.

The interindividual and intraindividual variations in the lifetimes of prostheses are extensive, but the differences within a prosthesis type are rather limited. In a group of 158 patients with 453 replacements, the lifetime of the Groningen prosthesis was on average 15.8 weeks (no range or median indicated).[8] A recent evaluation of the Provox 2 prosthesis revealed that the average lifetime in a population of 157 patients was 14.9 weeks with a range of 1 to 53 weeks, although in this study patients who required replacement within 2 months were put on an antimycotic regimen.[9] Regular long-term exposure of bacteria and yeasts to antibiotics and antimycotics unavoidably leads to the development of microbial resistance. As the overall 5-year survival rate of laryngectomized patients is 70%, the prescription of antimycotics to laryngectomized patients solely to prolong the prosthesis lifetime may strongly contribute to the development of antimycotic resistance among yeasts, as there exists only a limited number of antimycotics at present.[10,11] The development of increasing resistance against antibiotics and antimycotics has renewed scientific interest in probiotic bacteria.[12] The reported beneficial effects of probiotics include suppression of diarrhea, antitumor activity, stimulation of immunity, reduction of cholesterol levels, and relief of lactose intolerance.

A previous study examined the in vitro effects of various dairy products with probiotic bacterial strains on biofilm formation on voice prostheses, inspired by anecdotes suggesting that the consumption of buttermilk or Turkish yogurt may prolong the device lifetime.[13,14] In an in vitro artificial throat model, it

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PROBIOTIC BACTERIA USED

Strain	*pH**	*Growth Medium*	*Source*	*Probiotic Effects*
Streptococcus thermophilus B	4.0	M17, medium with 1% saccharose	Nizo, Ede, the Netherlands	Production of biosurfactants, inhibition of *Candida* adhesion[19]
Lactococcus lactis 53	5.3	BHI	Nutricia, Zoetermeer, the Netherlands	Antimycotic effects[20]
Lactococcus rhamnosus 744 (ATCC7469)	3.7	MRS	Nutricia	
Lactobacillus fermentum B54	6.3	MRS	Provided by Dr G. Reid, London, Canada	Biosurfactant release[21]
Enterococcus faecium 603	7.3	BHI	Nutricia	
Lactobacillus casei Shirota	4.3	MRS	Isolated from Yakult fermented milk drink (Yakult, Almere, the Netherlands)	Biosurfactant release[21]
Bifidobacterium infantis 420	5.1	Defined medium	Nutricia	
Lactococcus lactis cremoris SK11	5.9	BHI, with 0.5% lactose	Nizo	

M17 — medium with 1% saccharose added (Oxoid, Basingstoke, England); BHI — brain-heart infusion broth (Oxoid); MRS — de Man, Rogosa, and Sharpe agar (Merck, Darmstadt, Germany).

*Of diluted suspension as used in this study.

was demonstrated that buttermilk and Yakult fermented milk drink (milk drink containing *Lactobacillus casei* Shirota; Yakult, Almere, the Netherlands) had a strong inhibiting effect on biofilm formation. The artificial throat enables isolated single factors influencing biofilm formation to be studied separately, in contrast with in vivo studies.[15] Interestingly, none of the previous attempts working in the artificial throat with isolated pure cultures of bacteria or yeasts from explanted voice prostheses yielded biofilms on voice prostheses that resembled those found on used prostheses. In a teleological approach, inoculation with a mixture of bacteria and yeasts taken from an explanted Groningen voice prosthesis without identification of all strains and species included provided us with these clinically relevant biofilms.[16] A teleological approach as applied here has the scientific drawback that the inoculum is microbiologically less well defined, but because of the multitude of strains and species in oropharyngeal biofilms working in concert while colonizing a prosthesis in an unsterile environment with constant new microbial challenges, a reductionistic approach with isolated single strains is likely to fail to mimic the clinical situation.

The aim of this study was to investigate the antimycotic effects of probiotic bacteria on colonized voice prostheses. For some of the probiotic strains used in this study, antimycotic effects have been reported, while others are known to excrete biosurfactants that discourage pathogen adhesion to biomaterial surfaces.[17,18]

MATERIALS AND METHODS

Voice Prostheses. "Low-resistance" Groningen voice prostheses and Provox 2 voice prostheses were used in this study. The low-resistance Groningen voice prosthesis consists of a tube with 2 flanges with a semicircular slit of 145° in the "hat" of the esophageal flange, functioning as a 1-way valve. The Provox 2 voice prosthesis is a biflanged device. The valve is molded into one piece with the prosthesis and is supported by a radiopaque, fluoroplastic ring that is fastened in the shaft of the prosthesis. A diagrammatic comparison of both silicone rubber prostheses has been given by Leunisse et al.[15]

Probiotic Strains. The probiotic bacterial strains used are listed in the Table[19-21] together with the pH of the suspension, reported antimycotic or antibiotic effects, proven production of biosurfactants, the growth medium used, and the source of the strain. Each probiotic strain was cultured from agar plates in its specific growth medium for 24 hours at 37°C. This culture was used to inoculate a second culture that was grown for 16 hours. All strains were grown in ambient air, except for *Lactobacillus* strains, which were cultured in 5% carbon dioxide. Bacterial cultures were washed twice with demineralized water by centrifugation at 6,500*g* for 5 minutes. The bacterial pellets were resuspended in demineralized water at a concentration of 1×10^9 bacteria per milliliter.

Biofilm Formation in Artificial Throat. The voice prostheses were placed in 2 transparent modified Robbins devices, called artificial throats, described in detail before.[14] Each artificial throat was equipped with 3 Groningen and 2 Provox 2 voice prostheses. The third Groningen voice prosthesis was used for scanning electron microscopic examination. The previously isolated, total cultivable microflora from an explanted Groningen voice prosthesis, containing a va-

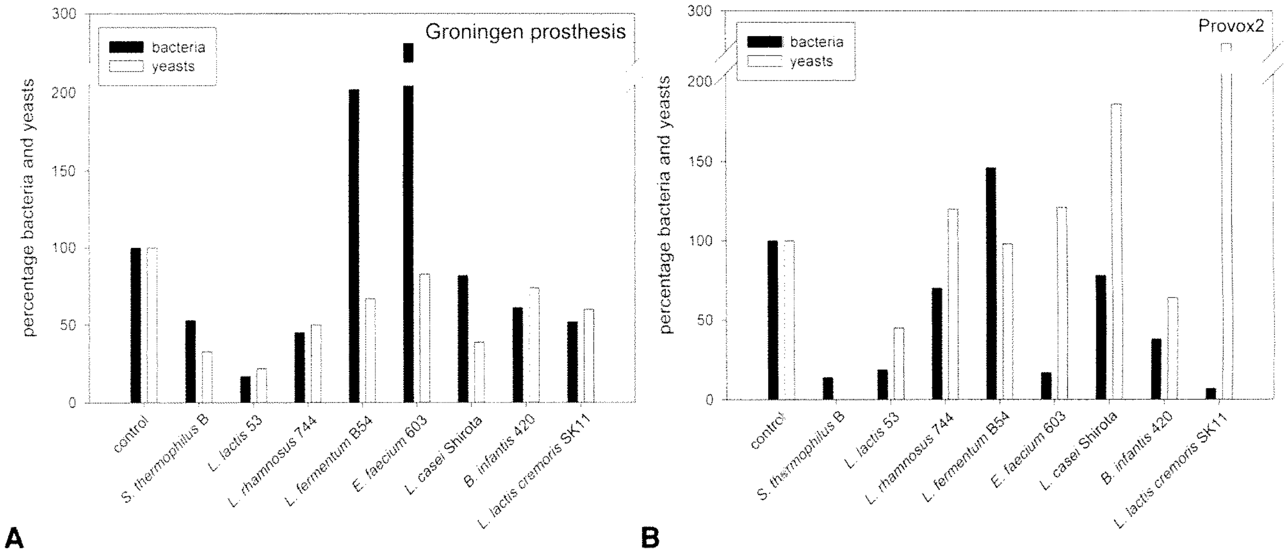

Fig 1. Percentages of bacteria and yeasts (see text for abbreviations) isolated from voice prostheses after biofilm formation in artificial throat and perfusion with various probiotic suspensions. As control, set at 100%, perfusion with phosphate-buffered saline solution was used. All experiments were done in duplicate. **A)** Groningen prostheses. **B)** Provox 2 prostheses.

riety of yeasts and bacterial strains, among which were *Candida albicans*, *Candida tropicalis*, and streptococcal and staphylococcal strains, was stored as a frozen stock. Aliquots were thawed at room temperature and cultured in a mixture of 30% brain-heart infusion broth (Oxoid, Basingstoke, England) and 70% defined yeast medium (per liter: 7.5 g glucose, 3.5 g $(NH_4)_2SO_4$, 1.5 g L-asparagine, 10 mg L-histidine, 20 mg DL-methionine, 20 mg DL-tryptophan, 1 g KH_2PO_4, 500 mg $MgSO_4.7H_2O$, 500 mg NaCl, 500 mg $CaCl_2.2H_2O$, 100 mg yeast extract, 500 μg H_3BO_3, 400 μg $ZnSO_4.7H_2O$, 120 μg $FE(III)Cl_3$, 200 μg $Na_2MoO_4.2H_2O$, 100 μg KI, and 40 μg $CuSO_4.5H_2O$) and were used to inoculate the artificial throats over 5 hours. Subsequently, a biofilm was allowed to grow on the voice prostheses for 3 days after the devices were filled with growth medium. On the 4th day, the devices were perfused with phosphate-buffered saline solution (10-mmol/L potassium phosphate and 150-mmol/L NaCl, pH 7.0) serving as a control or with a probiotic suspension, after which the prostheses were left in the moist environment of the drained artificial throats. This perfusion scheme was repeated 3 times per day over 9 days. At the end of each day, all artificial throats were filled with growth medium for half an hour and left empty during the night after draining. The experiments lasted 15 days in total and were performed at room temperature. The tracheal sides of the prostheses were left in ambient air without taking precautions to prevent infections from the air, similar to the situation with a stoma.

Evaluation of Biofilms. On the 15th day of the experiment, the prostheses were removed from the artificial throats after a final perfusion with phosphate-buffered saline solution. Subsequently, the biofilm was removed from the valve sides of the prostheses by scraping, and after sonication (60 seconds), it was serially diluted in reduced transport fluid (per liter: 0.9 g NaCl, 0.9 g $(NH_4)_2SO_4$, 0.45 g KH_2PO_4, 0.39 g $MgSO_4.7H_2O$, 0.45 g K_2HPO_4, 0.37 g ethylenediaminetetraacetic acid, and 0.2 g cysteine hydrochloride). Microbial compositions of the biofilms were evaluated by plating the serial dilution on MRS (de Man, Rogosa, and Sharpe) agar plates for yeasts and on blood agar plates for bacteria. After 3 days at 37°C in an aerobic incubator, the numbers of bacterial and fungal colony-forming units per milliliter were determined for each control prosthesis and for the prostheses in the artificial throats perfused with a probiotic bacterial suspension. Subsequently, the numbers of colony-forming units on the prostheses in the artificial throats perfused with a suspension of probiotic bacteria were expressed as a percentage relative to the number of colony-forming units found on the control prostheses. Consequently, potential variations in biofilm formation over different runs were monitored from the control throat and eliminated from the analysis.

Electron Microscopic Evaluation. Electron microscopy was only performed on Groningen voice prostheses, as biofilm features can be more easily discovered on Groningen than on Provox 2 prostheses. Biofilm-covered prostheses were flushed with 6.8% sucrose and 0.1-mol/L cacodylate buffer (pH 7.4), fixed and stained in 2% glutardialdehyde and 0.2% ruthenium red in 0.1-mol/L cacodylate buffer at 4°C, and flushed again. Post-fixation and staining was carried out in 1% osmium tetroxide and 0.2% ruthenium red in cacodylate buffer by gently shaking for 3 hours at

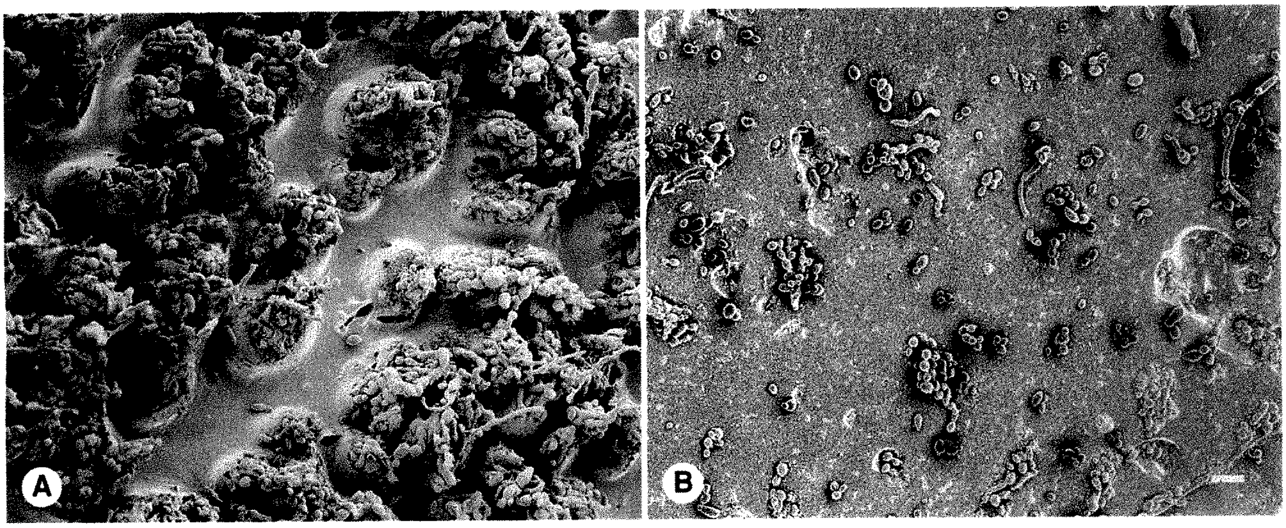

Fig 2. Scanning electron micrographs of Groningen voice prostheses removed from artificial throat after 15 days. Bar — 10 µm. **A**) Voice prosthesis taken from artificial throat perfused with phosphate-buffered saline solution. Biofilm and microbial ingrowth into silicone rubber are clearly visible. **B**) Voice prosthesis taken from artificial throat perfused with *Lactococcus lactis* 53 suspension.

room temperature. Buffer washes and dehydration involved the following rinsing procedures: 20 minutes in 6.8% sucrose in 0.1-mol/L cacodylate buffer; 3 × 10 minutes in bi-distilled water; 20 minutes each in 30%, 50%, and 70% ethanol; and 4 × 30 minutes in 100% ethanol. After critical-point drying with CO_2 for 4 hours, the specimens were mounted on scanning electron microscopy stubs and sputter-coated with gold-palladium (15 nm). The scanning electron microscopy observations were made with the JEOL 6301 with different magnifications at 15 to 25 kV.

RESULTS

Groningen Voice Prostheses. Exposure of prostheses to a suspension of *Lactococcus lactis* 53 had the strongest inhibitory effect on bacteria and yeast prevalence in the biofilms and reduced the percentages of bacteria and yeasts to 17% and 22%, respectively. Suspensions of *Streptococcus thermophilus* B, *Lactococcus rhamnosus* 744, *Bifidobacterium infantis* 420, and *Lactococcus lactis cremoris* SK11 yielded reductions in percentages of bacteria ranging from 45% (*L rhamnosus* 744) to 61% (*B infantis* 420), and they reduced the percentages of yeasts from 33% (*S thermophilus* B) to 74% (*B infantis* 420; Fig 1A). *Lactobacillus casei* Shirota reduced the amounts of bacteria and yeasts to 82% and 39%, respectively.

Provox 2 Voice Prostheses. Strong reductions in the percentages of bacteria by *L lactis cremoris* SK11 (7%), *S thermophilus* B (14%), and *Enterococcus faecium* 603 (17%) were observed, while *L lactis* 53 and *B infantis* 420 reduced the amounts of bacteria to 19% and 38%, respectively (Fig 1B). An *S thermophilus* B suspension fully eliminated all yeasts from the biofilm, but of all other probiotic suspensions, only those suspensions with *L lactis* 53 and *B infantis* 420 showed an inhibitory effect on yeast prevalence.

Scanning Electron Microscopy. Figure 2A shows a scanning electron micrograph of a Groningen voice prosthesis from an artificial throat perfused with phosphate-buffered saline solution. The surface shows a biofilm consisting of bacteria and yeasts with clear ingrowth into the silicone rubber. The surface of the voice prosthesis taken from the artificial throat perfused with an *L lactis* 53 suspension, on the other hand (Fig 2B), showed less biofilm, and ingrowth features can hardly be discerned.

DISCUSSION

Extending the lifetime of voice prostheses has been attempted for several years, as it would improve the quality of life of laryngectomized patients, as well as contribute to cost savings in health care. Clinical studies are hampered by the multitude of environmental and dietary factors influencing biofilm formation on voice prostheses; the artificial throat was introduced to enable the study of isolated single factors, as done here for probiotic bacterial suspensions.

At the end of the 19th century, the Russian scientist Metchnikoff studied probiotic bacteria and their effect on health and disease, but with the introduction of chemotherapy and penicillin in the 1940s, this line of research was not further pursued. As nowadays antimicrobial resistance is a growing source of concern in modern medicine, and health-improving, functional foods are gaining in popularity, the development of alternative prophylactic and therapeutic agents, including probiotics, has been revitalized.[12,17,18] Lactobacilli are one of the most well-known probiotic bacterial strains and play an important role in the maintenance of a healthy intestinal and urogenital tract.[21,22] Other bacterial strains known to have probiotic effects are lactococci, enterococci, *Bifidobacterium*, and streptococci. Some strains, however, may also have pathogenic traits and are therefore less suitable for clinical use. The mechanism by which probiotic bacteria exert their beneficial effects is not yet entirely clear. The Table shows that some strains are able to release biosurfactants, while others are known to have antimycotic effects and produce lactic acid or hydrogen peroxide. Also, competitive adhesion,[20,23-25] activation of the immune system,[19] and nutrient competition[26] have been suggested as mechanisms by which probiotic bacteria exert their beneficial effects.

The neopharynx in laryngectomized patients has major anatomic differences as compared with a healthy pharynx, including reduced salivary flow rates as a result of postoperative radiotherapy, and the presence of a foreign body, the voice prosthesis. Such changes in ecosystem are known to affect the microflora, causing a shift toward a more fungal-rich flora, as in denture wearers.[27] We found that yeast prevalence in voice prosthetic biofilms can be affected by introduction of probiotic bacteria in the ecosystem through the consumption of *L lactis* 53 when the foreign body is the Groningen prosthesis, whereas introduction of *S thermophilus* B has the greatest beneficial influence on yeast prevalence in biofilms on Provox 2 prostheses. Possibly, the specific geometry of the different prostheses influences which probiotic strain most successfully competes with the yeasts. Assuming yeast prevalence is associated with failure of the prosthesis, increases in prosthesis lifetime are to be expected from consumption of these probiotic strains, in line with the anecdotes circulating in patient populations.

Parker et al,[28] however, have suggested that besides yeasts, bacteria play an important role in prosthesis failure; streptococci have been associated with valve leakage, while enterococci have been held responsible for increased airflow resistance. Despite the effects of the probiotic bacteria on yeast prevalence in the biofilms, the probiotic strains apparently have a low tendency to colonize the voice prostheses themselves (Fig 1), as general bacterial prevalence in the voice prosthetic biofilms is reduced. This finding points to indirect interference mechanisms on the part of the probiotic bacteria, such as lactic acid and hydrogen peroxide production or the release of biosurfactants.

To summarize, it has been demonstrated that probiotic bacteria reduce biofilm formation on voice prostheses. Increased prosthesis lifetimes can be expected from carefully designed food supplements containing these bacteria. The bacteria can be administered in different forms, such as freeze-dried in sachets or in a dairy product.

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NEUROMUSCULAR DISORDERS PRESENTING AS CONGENITAL BILATERAL VOCAL CORD PARALYSIS

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Congenital bilateral vocal cord paralysis (BVCP) can be associated with an underlying neuromuscular disorder, and may present before other features of the neuromuscular disorder become apparent. All infants less than 12 months of age presenting with BVCP between July 1987 and July 1999 at the Royal Children's Hospital, Melbourne, in whom a neuromuscular disorder was subsequently diagnosed were followed. Three children in whom BVCP was diagnosed soon after birth and before recognition of an underlying neuromuscular disorder were identified. All presented with upper airway obstructive symptoms at birth, had a diagnosis of bilateral abductor vocal cord paralysis made at awake flexible laryngoscopy, and had no underlying structural laryngeal abnormality on microlaryngoscopy and bronchoscopy. Two children required a tracheostomy, and 1 child was weaned from nasopharyngeal continuous positive airway pressure after 3 weeks. Subsequent neuromuscular symptoms were recognized between 4 months and 7 years later, leading to diagnoses of facioscapulohumeral myopathy, spinal muscular atrophy, and congenital myasthenia gravis. In each case, the prognosis for recovery from symptoms related to BVCP reflected that of the underlying neuromuscular disorder. This experience suggests that congenital BVCP may be a feature of an unrecognized neuromuscular condition. This possibility should be considered particularly in the presence of associated neurodevelopmental or neuromuscular dysfunction, or in cases in which BVCP is progressive.

KEY WORDS — congenital bilateral vocal cord paralysis, neuromuscular disorder.

INTRODUCTION

Significant upper airway obstruction presenting immediately after birth should raise the possibility of congenital bilateral vocal cord paralysis (BVCP). However, arriving at a diagnosis of congenital BVCP only marks the beginning of a long process for caregivers and physicians, and important questions such as the severity of the condition, the underlying cause, the prognosis for recovery, and the best therapeutic strategies need to be addressed.

The prognosis for recovery of vocal cord function depends on the underlying cause of the congenital BVCP.[1] After excluding structural laryngeal abnormalities that limit vocal cord mobility, the causes of congenital BVCP that need to be considered are birth trauma or asphyxia, neuromuscular disorders, and central nervous system anomalies, most commonly the Arnold-Chiari malformation. Other structural neurologic abnormalities have also been described, including hydrocephalus unrelated to the Arnold-Chiari malformation, hypoplasia of cranial nerve motor neurons, and cerebral agenesis.[2] In some cases, the cause of congenital BVCP may not be readily apparent.

In their review of the etiology of BVCP, Holinger et al[2] found that in 71 of 114 cases of congenital BVCP, no cause was determined. Associated anomalies were also present in 32 of these children, which included neurologic, cardiac, and laryngeal abnormalities. Emery and Fearon[3] found that in 10 of 19 children with congenital vocal cord paralysis, the cause was unknown, and 5 of these had other anomalies. As has been suggested,[1] it would appear that in a significant proportion of cases of congenital BVCP, no cause may be found.

The high proportion of idiopathic congenital vocal cord paralysis in these reports by Holinger et al[2] and Emery and Fearon[3] may be partly due to the lack of the sophisticated laboratory, electrophysiological, and imaging studies that are available today. However, a modern protocol for the investigation of congenital BVCP has yet to be established. A previous series of neonates evaluated by awake flexible laryngoscopy[4] has highlighted the importance of this method in the investigation of congenital BVCP. Laryngeal electromyography findings in idiopathic congenital BVCP[1] and animal studies of posterior cricoarytenoid muscle activity,[5] as well as the identification of posterior cricoarytenoid motoneurons in the medulla,[6,7] may further contribute toward our understanding of this condition.

Congenital BVCP itself has also been associated with manifest findings indicative of a more widespread neuromuscular disorder.[8] As the primary cause of congenital BVCP, neuromuscular disorders include a range of conditions that affect the anterior

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DETAILS OF CASES

	Patient 1	*Patient 2*	*Patient 3*
Sex	Female	Male	Male
Presenting airway symptoms	Respiratory distress requiring immediate intubation	Inspiratory stridor without respiratory distress	Inspiratory stridor and respiratory distress
Time of airway symptom presentation	Birth	Birth	Birth
Airway investigations	Awake flexible laryngoscopy, microlaryngoscopy and bronchoscopy, laryngeal electromyography	Awake flexible laryngoscopy, microlaryngoscopy and bronchoscopy	Awake flexible laryngoscopy, microlaryngoscopy and bronchoscopy
Airway diagnosis	Complete bilateral abductor paralysis	Partial bilateral abductor paralysis	Complete bilateral abductor paralysis
Intervention	Tracheostomy at 3 d	Tracheostomy at 8 mo	Nasopharyngeal CPAP from birth to 3 wk
Presenting neuromuscular symptoms	Left facial weakness initially thought to have resolved in weeks, but subsequently recognized as facial muscle hypoplasia, mild developmental delay, upper arm and shoulder weakness	Gross motor delay, progressive hypotonia and weakness, tongue fasciculations	Feeding difficulties, ptosis, facial weakness, gross motor delay and hypotonia
Time of neuromuscular symptom presentation	Facial weakness at birth, other symptoms of insidious onset and progressively worsening until age 7 y	Age 11 mo	Age 4 mo
Neuromuscular investigations	CT: mildly dilated ventricles MRI: normal examination EMG: myopathic features Muscle biopsy: moderate degree of type 2 fiber atrophy	MRI: normal examination EMG: myopathic features Muscle biopsy: duality of fiber diameters, type 1 hypertrophy DNA: SMN (survival motoneuron) gene deletion not detected	MRI: mildly distended occipital horns of lateral ventricles, abnormal white matter signals Muscle biopsy: neuropathy, type 1 fiber atrophy DNA: no genetic abnormality
Neuromuscular diagnosis	Facioscapulohumeral myopathy	Spinal muscular atrophy	Congenital myasthenia gravis
Subsequent course	Acquired subglottic stenosis, failed laryngotracheoplasty and first-stage arytenoid lateralization, tracheostomy dependence, minimal vocal cord abduction	Failed posterior cordotomy, tracheostomy dependence due to worsening respiratory function, minimal purposeful vocal cord abduction	Resolution of vocal cord palsy with medical management of myasthenia
Duration of follow-up	7 y 9 mo	4 y 3 mo	3 y 9 mo

CPAP — continuous positive airway pressure, CT — computed tomography, MRI — magnetic resonance imaging, EMG — electromyography.

horn cells, neuromuscular junction, and muscle. These disorders can present with BVCP in addition to weakness and hypotonia. However, the onset of BVCP may occur before or after other features of a neuromuscular disorder become apparent. While symptoms of BVCP may not always be present at birth, neither may the recognition of the neuromuscular disorder that in some cases may be preceded by BVCP. We present our experience with 3 children in whom BVCP was diagnosed soon after birth, and before recognition of an underlying neuromuscular disorder.

METHODS

All infants less than 12 months of age presenting with BVCP from July 1987 to July 1999 at the Royal Children's Hospital in whom a neuromuscular disorder was subsequently diagnosed were followed, and their case histories were reviewed. Children were included in the study if the diagnosis of BVCP was made before the recognition of an underlying neuromuscular disorder, although some patients may have had some evidence of a neuromuscular disorder that was unrecognized at the time of diagnosis of BVCP.

RESULTS

Clinical details of 3 children with BVCP associated with a subsequently diagnosed neuromuscular disorder are listed in the Table. All presented with upper airway obstructive symptoms at birth, received a diagnosis of bilateral abductor vocal cord paralysis at awake flexible laryngoscopy, and had no underlying structural laryngeal abnormality seen on microlaryngoscopy and bronchoscopy. A tracheostomy was required in 1 child with complete BVCP and in another child with partial BVCP, on the third day and in the eighth month of life, respectively. The third child, who had complete BVCP at birth, was weaned from nasopharyngeal continuous positive airway pressure at 3 weeks. Subsequent neuromuscular symptoms were recognized in these children 4 months to 7 years later, and investigations led to the diagnoses of facioscapulohumeral myopathy, spinal muscular atrophy, and congenital myasthenia gravis. The prognosis for recovery from symptoms related to BVCP in each case reflects that of the underlying neuromuscular disorder.

DISCUSSION

Three children with congenital BVCP are described, all of whom subsequently received diagnoses of neuromuscular disorders. Previous reports of vocal cord paralysis preceding the diagnosis of neuromuscular disorders in children have included cases of acute spinal muscular atrophy[9] and subacute necrotizing encephalomyelopathy or Leigh disease.[10]

The neuromuscular disorder may become readily apparent soon after diagnosis of BVCP, as in the case of patient 3, with congenital myasthenia, in whom medical therapy may have avoided the need for tracheostomy. A similar case has been reported of myasthenia gravis presenting with acute stridor due to BVCP in a 46-year-old man.[11] In contrast, the underlying neuromuscular disorder may not be fully appreciated for years, as in the case of patient 1, in whom a specific diagnosis of facioscapulohumeral myopathy was only made for progressively worsening neurologic developmental symptoms at age 7. Wu et al[12] reported the case of a 53-year-old man in whom vocal cord paralysis necessitating a tracheostomy preceded any other neurologic and autonomic manifestations by 2 years, until extrapyramidal and cerebellar signs with autonomic failure led to magnetic resonance imaging diagnosis of severe brain stem and cerebellar atrophy.

In cases in which no recognizable cause for BVCP can be found, no prognostic indicators are available at the moment for recovery of vocal cord function. The severity of BVCP is usually gauged by the degree of upper airway obstruction present and the need for tracheostomy. Generally, if tracheostomy is not necessary in the neonatal period, progressive improvement in vocal cord function will occur without the need for subsequent intervention. Conversely, when tracheostomy is required in a neonate, tracheostomy dependence may persist for a number of years, and any subsequent improvement in vocal cord movement may take years and may be only partial.[13] While this may be true for idiopathic congenital BVCP, an underlying neuromuscular disorder must be considered when the degree of vocal cord dysfunction increases with age. Patient 2, with partial vocal cord paralysis, developed tracheostomy dependence in the postneonatal period. The index of suspicion for an underlying neuromuscular disorder should be very high in this clinical situation.

Besides causing impairment of vocal cord function, a neuromuscular disorder may also affect respiratory muscle function. The clinical severity of BVCP may therefore be magnified by the presence of associated poor respiratory effort. Both mechanisms were involved in patient 2, with spinal muscular atrophy, the former explaining the initial need for tracheostomy, and the latter explaining the subsequent continuing tracheostomy dependence despite the presence of some purposeful vocal cord abduction.

The need for both structural and functional assessment of the neonatal upper airway, the importance of identifying an underlying cause for congenital BVCP, and the importance of recognizing the association between congenital BVCP and other congenital abnormalities remain the cornerstones of the current approach to the evaluation of the newborn with BVCP.[13] Our experience suggests that congenital BVCP may be an early warning of an unrecognized neuromuscular condition. This possibility should be considered, particularly in the presence of associated neurodevelopmental or neuromuscular dysfunction, or when BVCP is progressive.

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AGE DEPENDENCE OF LARYNGEAL CHEMOREFLEX IN PUPPIES

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Previously collected data have indicated that the laryngeal chemoreflex (LCR) response is exaggerated during a critical period of postnatal development in several experimental animals. These animals had fewer anatomic and physiological similarities to humans than do puppies. This investigation of the LCR in 14 anesthetized puppies was undertaken to determine age-related differences in the response to stimulation of the supraglottic laryngeal mucosa by saline solution, distilled water, cow's milk, and acid at pH 1.0. The dogs were divided into 3 age groups: group 1 consisted of 4 dogs that were 2 weeks old, and in groups 2 and 3 there were 5 puppies each, of 4 and 6 weeks of age, respectively. The LCR response (laryngospasm, apnea, respiratory depression, and bradycardia) was found in the puppies only after stimulation of the laryngeal mucosa with acid at pH 1.0, and it was more easily achieved in the 4- and 6-week age groups than in the 2-week group. These findings suggest that the LCR is an age-dependent response that appears in dogs only after 2 weeks of age. The important implication of this finding is that postnatal neural maturation may influence the laryngeal reflex in humans to some extent.

KEY WORDS — laryngeal adductor reflex response, laryngeal chemoreflex, postnatal neural maturation, superior laryngeal nerve.

INTRODUCTION

Laryngeal reflexes play an important role in respiratory control and pulmonary protection from aspiration. Chemical or mechanical stimulation of the tactile receptors on the supraglottic mucosa or direct electrical stimulation of the superior laryngeal nerve (SLN) may result in either or both of two responses. The first response, the laryngeal adductor reflex response, is a polysynaptic brain stem response that causes glottic closure.[1-3] The second response, the laryngeal chemoreflex (LCR) response, results in centrally mediated apnea, hemodynamic changes, and swallowing.

Studies on the LCR response in animal subjects have demonstrated postnatal maturational variations.[4-7] It appears that the magnitude of the LCR response is exaggerated during a critical period of postnatal development. Also, the response has been shown to be influenced by hypoxia and stimulation with various substances.[4,8] The current study of LCR in immature dogs was undertaken to investigate age-related differences in susceptibility to such stimulation.

The dog may represent a more valid experimental subject than other animals for the neurophysiological study of laryngeal reflexes, in that its larynx possesses a high degree of anatomic and physiological similarity to that of a human.[9] It is thus expected that the results of such studies as this will be instructive and very likely applicable to the study of the human LCR. Hence, we designed an LCR study to use 3 groups of puppies (2, 4, and 6 weeks old) to 1) determine whether dogs are appropriate animals to use as subjects for research on the LCR, 2) compare the effects of differing stimulants in elicitation of the LCR, and 3) compare LCR responses in subjects of different ages.

MATERIALS AND METHODS

Preparation and Setup of Subjects for LCR Experiment. Fourteen puppies, each weighing between 3.5 and 6 kg, were used for the experiment. They were divided into 3 age groups. In group 1, there were 4 puppies 2 weeks of age, and groups 2 and 3 consisted of 5 puppies each, 4 and 6 weeks of age, respectively. Each dog was premedicated with intramuscular atropine sulfate at a concentration of 0.04 mg/kg. Anesthesia was induced with intramuscular ketamine hydrochloride at a concentration of 10 mg/kg and maintained with administration of small amounts of the same drug via a femoral venous catheter every 30 minutes.

With the subject in the supine position, we incised its anterior neck skin at the midline from the level of the hyoid bone down to the suprasternal notch, thus exposing the larynx and cervical trachea. The trachea was entered below the second ring, and a low tracheotomy was made. A balloon-cuffed endotracheal tube, 3.5 to 4.0 mm in diameter, was inserted

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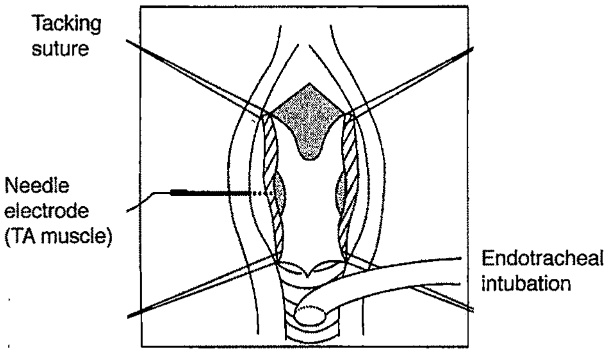

Fig 1. Preparation and setup of puppy for laryngeal chemoreflex experiment. Transverse incision of thyrohyoid membrane was made, and laryngofissure was performed at midline from notch of cartilage down to cricothyroid membrane. Each thyroid ala was tacked with suture ties, and inner side of larynx was exposed. Bipolar, concentric needle electromyographic (EMG) electrodes were placed into right thyroarytenoid (TA) muscle by passing them through right thyroid cartilage, into belly of muscle.

into the distal trachea. Transverse incision of the thyrohyoid membrane was made with care, so as not to damage either internal branch of the SLN. A laryngofissure was performed in the midline from the notch of the cartilage down to the cricothyroid membrane, with care taken not to damage either vocal cord. Each thyroid ala was tacked with suture ties, and the inner side of the larynx was exposed. The LCR response was checked by monitoring the respiration rate, heart rate, and thyroarytenoid (TA) muscle electromyographic (EMG) readings by means of the MP100A Data Acquisition System (Biopac Systems, Inc, Santa Barbara, California) and Biopac's Acqknowledge software.

Bipolar, concentric needle EMG electrodes were inserted into the right TA muscle by passing them through the right thyroid cartilage and into the belly of the muscle (Fig 1). In this way, the mucosa over the muscle was not violated. Reference and ground electrodes were placed into the strap muscles. All of the EMG electrodes were connected to and monitored by a Nicolet Viking IV Plus EMG machine (Nicolet Biomedical, Inc, Madison, Wisconsin). The EMG response was recorded by an averaging computer with a 60-Hz filter and a bandpass of 100 to 300 Hz. The Nicolet Viking IV Plus was connected to a UIM100A Universal Interface Module and an MP100A Data Acquisition Unit, so the EMG results were collected into the main MP100A unit. The TSD101A Respiratory Effort Transducer, attached tightly to the abdominal wall of each of the dogs, measured the respiratory rate. The TSD101A was connected to an RSP100 Respiration Pneumogram Amplifier, and the data gathered were fed into the UIM100A Universal Interface Module and the MP100A Data Acquisition Unit. Surface electrodes of an electrocardiograph (ECG) were attached to the right and left subcostal areas and the left leg, and the heart rate was monitored via an ECG and its data were fed into the MP100A system.

Level of Anesthesia. A mixture of isoflurane and oxygen at a concentration of 2.5 L/min was administered via the tracheotomy site, and the level of anesthesia was achieved by controlling the above gases. Level 3 anesthesia was maintained throughout the experiment, and at that level, puppies have spontaneous respiration, an absence of corneal reflex, and, upon mechanical stimulation with a cotton-tipped applicator, neither a cough reflex nor changes in respiratory or laryngeal muscles. The concentration of inspired isoflurane needed to reach the proper level of anesthesia varied with each puppy, but remained within a range of 0.5% to 1.0%.

Methods of Stimulating Supraglottic Mucosa and Monitoring Responses. After the proper level of anesthesia was reached, attempts were made to induce an LCR by instillation into the supraglottic mucosa of the following stimulants: a physiologic saline solution, distilled water, cow's milk, and a strong acid solution. The experiment was undertaken twice on each dog with each stimulating substance. Acid solutions at pH 2.0, 1.5, and 1.0 were each instilled into the endolarynx, but only the acid solution at pH 1.0 induced a stable LCR response, so the pH 1.0 solution was selected for the experiment. The acid solutions were made by titration with 0.9% sodium chloride to pH level 1.0 with 1N hydrochloric acid. Benchtop pH/ISE meters (Orion Research Inc, Cambridge, Massachusetts) were used in the pH measurements. About 3 to 4 mL of each test solution was placed into the larynx. After each test material was instilled, at least 60 seconds was allowed for both EMG and visual confirmation of laryngospasm. The amount administered filled the laryngeal basin, providing contact with the solution to all of the laryngeal mucosa. After each instillation, the solution was suctioned, and the endolarynx was rinsed 3 times with phosphate-buffered saline solution at pH 7.4. After irrigation, a return to baseline EMG activity was seen in each case. To regain the normal physiologic state, an interval of at least 10 minutes was allowed between stimulations.

Samples for blood gas analysis were obtained from the right femoral artery just before chemical stimulation, and were checked to confirm that the arterial carbon dioxide tension was below 40 mm Hg and the arterial oxygen tension was above 115 mm Hg, that is, in the normoxic state.[7] After stimulation of the laryngeal mucosa, TA muscle contraction was detected both by EMG study and observation with the

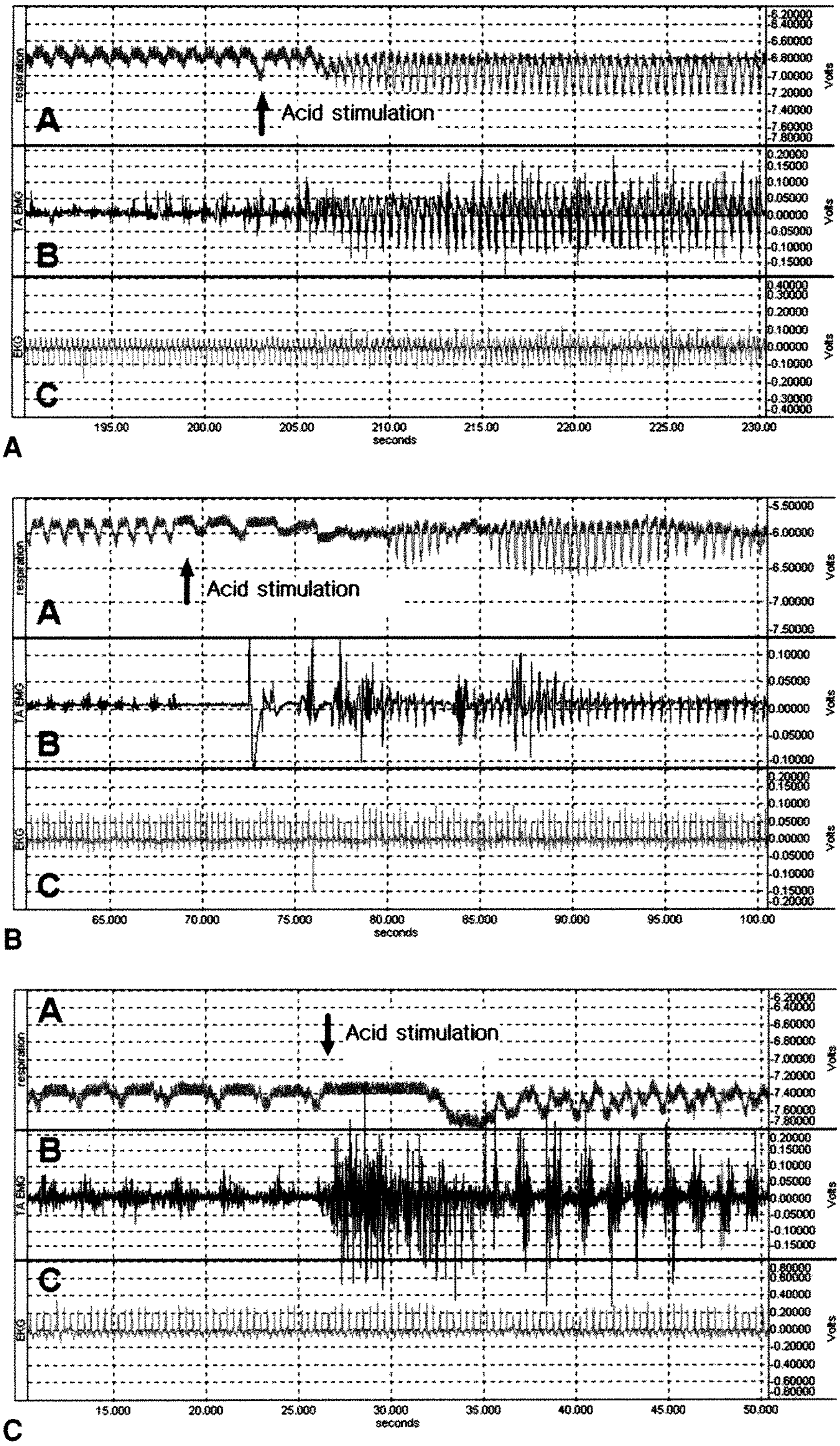

Fig 2. Polygraph tracings of puppies' responses to acid solution at pH 1.0. A — respiration tracing; B — TA muscle EMG tracing; C — heartbeat tracing. Arrow shows stimulation point. X-axis is time-scaled, and time interval between scales is 5 seconds. Y-axis shows degree of response. **A)** Two-week-old puppy. As can be seen, there was no initial depression of respiration, but there was elevation of respiratory rate. There was no laryngospasm or strong contraction as assessed by naked eye or EMG study. This response was termed type 1 muscle contraction. Heart rate was reduced, but it did not show marked difference from prestimulation state. **B)** Four-week-old puppy. Respiration was moderately depressed initially, and then respiratory rate was immediately elevated. TA muscle showed no laryngospasm, but strong contraction was noted initially and was followed by some large waves recorded on EMG study. We termed this type 2 muscle contraction. There was decrease in heart rate, but again, there was no marked difference from prestimulation state. **C)** Six-week-old puppy. Respiration was depressed for more than 5 seconds initially, and this depression was followed by compensatory elevation of respiratory rate. Laryngospasm of TA muscle was detected by naked eye and EMG study, and this was categorized as type 3 muscle contraction. Heart rate showed some reduction, but there was no marked change.

naked eye. The data, including the TA muscle EMG data, respiratory rate, and heart rate, were collected and analyzed by use of the MP100A system and Biopac's Acqknowledge software. The respiratory status was classified as apnea or the presence or absence of respiratory depression. Apnea was defined as a cessation of breathing for more than 5 seconds. Respiration and heart rates were recorded for 60 seconds after the stimulation. Once this initial protocol was completed, the SLN on each side was identified and sectioned. The acid instillation experiments were repeated in an identical manner to determine whether the LCR response could be elicited in the absence of supraglottic afferent innervation.

TABLE 1. RESPIRATION CHANGES IMMEDIATELY FOLLOWING STIMULATION WITH ACID SOLUTION AT pH 1.0

Age of Dogs (wk)	n	Apnea (>5 s)	Respiratory Depression +	Respiratory Depression −
2	8	0	0	8
4	9	4	5	0
6	10	3	7	0

Analysis of Results. The LCR response was induced in subjects in 3 age groups (2 weeks, 4 weeks, and 6 weeks of age) by the use of various stimulating substances, and data analysis after the LCR experiments was performed by the naked eye, an MP100A data acquisition unit, and Biopac's Acqknowledge software. Statistical analysis was made by use of a Wilcoxon signed-ranks test, which allowed analysis of both direction and magnitude of response, by means of comparison of prestimulation and post-stimulation states.

RESULTS

After stimulation with physiologic saline solution, distilled water, and cow's milk, no LCR response was noted in any age group. Strong acid solutions at pH 2.0 or 1.5 did not evoke a stable LCR response, but an acid solution at pH 1.0 induced meaningful, stable results. One subject in the 4-week-old group showed an unstable response after acid stimulation during the second experiment, so this case was omitted. Thus, the total numbers of LCR experimental results were 8, 9, and 10 observations in the first, second, and third age groups, respectively.

Once the initial protocol was completed, the internal branch of the SLN on each side was identified and sectioned. The acid instillation experiments were repeated in an identical manner after the sectioning of the SLN, and it was confirmed that the LCR response had not been elicited in the absence of supraglottic afferent innervation.

Figure 2 shows the LCR responses in this study. The changes of respiration and heart rate and the various types of TA muscle contraction are noted below.

Respiration Changes. After acid stimulation, there were various types of initial respiratory depression, followed by an elevation of respiratory rate (Fig 2). In the 2-week-olds, respiration was initially depressed minimally or not at all, but an increase of respiratory rate eventually occurred. We noted 4 cases of apnea and 5 cases of respiratory depression in the 4-week age group, with a mean duration of apnea of 8.1 ± 1.4 seconds. Three cases of apnea and 7 cases of respiratory depression were noted in the oldest subject group, and the mean duration of apnea was 9.2 ± 1.1 seconds (Table 1). These changes from prestimulation states were of statistical significance in all 3 age groups (Table 2).

TABLE 2. RESPIRATION CHANGES FOLLOWING RESPIRATORY DEPRESSION AFTER STIMULATION WITH ACID SOLUTION AT pH 1.0

Age of Dogs (wk)	n	Respiration (breaths/min) Before Stimulation	Respiration (breaths/min) After Stimulation	p
2	8	40.5 ± 2.1	69.5 ± 3.3	.011
4	9	34.1 ± 0.7	68.4 ± 3.2	.008
6	10	31.5 ± 1.8	66.9 ± 15.5	.005

TABLE 3. HEART RATE CHANGES FOLLOWING STIMULATION WITH ACID SOLUTION AT pH 1.0

Age of Dogs (wk)	n	Heart Rate (beats/min) Before Stimulation	Heart Rate (beats/min) After Stimulation	p
2	8	196.0 ± 6.8	186.4 ± 8.5	.18
4	9	185.3 ± 7.0	182.6 ± 9.9	.35
6	10	151.8 ± 4.9	142.4 ± 4.7	.07

Heart Rate Changes. After stimulation of the laryngeal mucosa with strong acid solution, the heart rates declined in every age group, but not to a statistically meaningful degree (Table 3).

TA Muscle Contractions. Three types of TA muscle contraction pattern were found, which were designated types 1, 2, and 3. Type 1 showed no laryngospasm, although strong contraction was detected by EMG testing. Type 2 showed no laryngospasm, but a strong contraction was immediately detected, followed by some large waves registering on the EMG test. Type 3 showed laryngospasm that was visible to the naked eye and also detected via EMG testing (Fig 2).

Five cases of type 1 and 3 cases of type 2 were noted in the youngest group. Two cases of type 1, 6 cases of type 2, and 1 case of type 3 occurred in 4-week-old dogs, and 2 cases of type 2 and 8 cases of type 3 were observed in the 6-week-old cohort (Table 4). Laryngospasm was not noted in the 2-week-olds, but it was seen in 1 of the 4-week-olds and in 8 of the 6-week-olds. The mean latency time before elicitation of TA muscle contraction was 2.4 ± 0.2 seconds in the youngest group, 4.6 ± 0.9 seconds in the

TABLE 4. TYPES OF THYROARYTENOID MUSCLE CONTRACTION FOLLOWING STIMULATION WITH ACID SOLUTION AT pH 1.0

Age of Dogs (wk)	n	Type 1	Type 2	Type 3
2	8	5	3	0
4	9	2	6	1
6	10	0	2	8

See text for definition of types of contraction.

TABLE 5. PEAK-TO-PEAK AMPLITUDE DIFFERENCES OF THYROARYTENOID MUSCLE CONTRACTION FOLLOWING STIMULATION WITH ACID SOLUTION AT pH 1.0

		Peak-to-Peak Amplitude Difference (mV)		
Age of Dogs (wk)	*n*	*Before Stimulation*	*After Stimulation*	*p*
2	8	125.0 ± 43.6	1,260.6 ± 511.0	*
4	9	75.6 ± 15.8	1,285.7 ± 795.0	.018
6	10	74.4 ± 13.3	1,030.0 ± 667.1	.005

*Contraction observed in only 3 cases.

4-week-old group, and 2.2 ± 0.7 seconds in the 6-week-old group. The duration of laryngospasm or type 2 strong contraction was 6.5 ± 1.4 seconds in the 2-week-old group, 7.5 ± 0.9 seconds in the 4-week-old group, and 6.9 ± 1.0 seconds in the 6-week-old group.

The peak-to-peak amplitude differences of TA muscle contraction increased statistically significantly ($p < .02$) in the 4- and 6-week-old groups (Table 5).

DISCUSSION

The physiological functions of the laryngeal reflex are generally classified into respiration, phonation, and protection of the lower respiratory tract and lungs from harmful substances. The LCR is a well-known type of laryngeal reflex, and its response involves centrally mediated apnea, laryngospasm, swallowing, and hemodynamic changes such as hypertension and tachycardia.[4-6,10,11] Even though weak reflexive glottic closure may also be evoked by stimulation of the trigeminal nerve or glossopharyngeal nerve,[3] the LCR pathway initiates powerful reflexive glottic closure in the following manner. After mechanical or chemical stimulation of the glottic or supraglottic mucosa, the internal branch of the SLN is activated and the stimulus is transferred via vagal afferent fibers into the nodose ganglion and then the nucleus solitarius in the brain stem. After that, laryngeal adductor motor neurons in the nucleus ambiguus and reticular formation are activated, and glottic closure and protection of the lower respiratory tract result.[12-21] It is clear that the LCR pathway has an afferent limb passing through the internal branch of the SLN, because the LCR response disappears after sectioning of the vagus nerve or of the SLN itself.[22]

The laryngeal reflex is primitive, but is important in that it protects the individual from inhalation of harmful substances. When this laryngeal reflex is exaggerated, it may be responsible for disorders such as spasmodic dysphonia, idiopathic laryngospasm, apnea of infancy, reflex apnea, stuttering, sudden infant death syndrome, and so on.[3,4,12,23-26]

The LCR response has been observed in several experimental subjects, including preterm human infants,[27] newborn lambs,[23,28,29] piglets,[6,23,30-33] adult dogs,[34] infant monkeys,[35] infant cats,[5] infant cows,[4] and infant rabbits,[36] but LCR experiments involving puppies apparently have not previously been conducted. These studies on the LCR response have demonstrated postnatal maturational variations, and it seems that the magnitude of the LCR response is exaggerated during a critical period of postnatal development but is progressively reduced as the subjects get older.[4-7] However, rapid development of laryngeal function has been noted in 4- to 6-week-old puppies.[37] Sasaki[12] similarly reported a period of laryngeal hyperexcitability from 7 to 11 weeks of age. Other studies[38,39] report that the esophagolaryngeal adductor reflex has a period of hyperexcitability from 8 to 19 weeks of age, during which time laryngeal adduction and laryngospasm also occur, even though the esophagolaryngeal adductor reflex is a unique reflex and quite dissimilar to the LCR. Fatal apnea has been noted in piglets less than 3 weeks of age,[6] and an increase in apnea has been reported in cats of the same age range[5]; thus, it seems that the periods of laryngeal hyperexcitability among the various experimental subject animals vary to a large degree. Variations in LCR response in individual animals were noted in the current study, even though the LCR response was checked under tightly controlled experimental conditions. These disparities may have been caused by slight differences in the attachment positions of the EMG electrodes or by differences in individual sensitivity. Also, the LCR is a complex response that can be influenced by a variety of external factors.[40]

Puppies were selected as the subjects of our experiments, first of all, because the larynx of a dog has a high degree of anatomic similarity to that of a human. Second, the latency time of laryngeal adduction in dogs is 16 to 18 ms, so it is relatively near the 25- to 27-ms latency period in humans.[9] Third, in cats, the period of latency of laryngeal adduction after contralateral stimulation of the SLN is longer than that of ipsilateral stimulation of the SLN, and about twofold stimulation intensity is needed in contralateral stimulation to get the same degree of action potentials as in ipsilateral stimulation.[41] Cats have a crossed adductor reflex as described above, but dogs and humans merely have or do not have a crossed adductor reflex, respectively. Thus, dogs and humans have greater neurophysiological similarity of the larynx.[9]

Substances known to induce the LCR response in other animals are distilled water, cow's milk, physiologic saline solution, strong acid solution, fetal tracheal fluid, and amniotic fluid.[4,23,29,34] Some researchers have suggested gastric acid as a possible cause of laryngospasm[34] or sudden infant death syndrome in the gastroesophageal reflux patient. Thus, the substances used for stimulation in the present study included strong acid solution at pH 1.0, 1.5, and 2.0, physiologic saline solution, distilled water, and cow's milk. How and which chemical stimulants affect the chemical receptors in the upper respiratory tract of humans is not yet clear, but another study has reported that the effects may be related to chloride ion deficiency, and are similar to those in puppies.[27]

Lanier et al[4] have reported that the magnitude of the LCR response and apnea increased with decreasing concentrations of sodium chloride and decreasing pH of stimulants. In another study, laryngospasm was noted in adult dogs after stimulation with strong acid solution at less than pH 2.5.[34] Physiologic saline solution only provokes mechanical stimulation of the laryngeal receptors, but water or distilled water induces a more powerful response because of additional chemical stimulation.[27] Only strong acid solution at pH 1.0 induced the LCR response, and the response was stronger in the 6-week-old puppies, in the current study. As in the research of Loughlin et al,[34] discussed above, neither physiologic saline solution nor distilled water induced laryngospasm. The magnitude of the LCR response of the puppies in this study was smaller than that of the adult dogs in the Loughlin et al study. When we compare our results with the report of Bauman et al[38] that esophagolaryngeal adductor reflex laryngospasm was easily induced in 8- to 19-week-old dogs, we believe that the fact that the LCR response was not noted in the 2-week-olds but had become apparent in the 6-week-olds indicates that the central nervous system and neuronal maturation of the laryngeal reflex arc is under way, although incomplete, in dogs 6 weeks of age. Thus, further consideration and research are called for in this area.

Some investigators have reported that LCR responses disappear and the normal physiologic state returns under normoxic conditions ($PaO_2 > 115$ mm Hg),[7] but that in a hypoxic state ($PaO_2 < 80$ mm Hg) the apnea induced by the LCR response increases to the degree that some subjects need resuscitation or die.[4,7,8] Others have reported, however, that apnea is meaningfully reduced under hypoxic conditions according to physiologic principles,[6,32] and yet others have reported that hypoxia has no influence on the degree of LCR response.[30] Efforts were made in the present study to ensure that the subjects never fell into a hypoxic state, and they involved continuous administration of an adequate concentration of oxygen (2.5 L/min) while the puppies were under general anesthesia, and obtaining samples for blood gas analysis from the right femoral artery just before every chemical stimulation so as to determine whether the arterial carbon dioxide and oxygen tensions were normal or not.

In general, laryngeal reflexes are greatly influenced by the depth of general anesthesia, so we maintained level 3 anesthesia throughout the experiment, and at that anesthetic level, the laryngeal mechanoreflex is absent, although the LCR is present. At that level, puppies have spontaneous respiration, an absence of corneal reflex, and, upon mechanical stimulation with a cotton-tipped applicator, neither cough nor respiratory nor laryngeal muscle changes. Also, when lidocaine, a local anesthetic agent, was systemically administered intravenously, it did not influence the laryngeal reflexes.[31] When, however, the lidocaine was administered locally into the endolarynx, both the laryngeal mechanoreflex and the LCR disappeared, because of the drug's direct blocking effect on laryngeal receptors, even though the laryngeal reflexes have a centrally mediated reflex arc.[31]

Some studies[7,34] have used EMG measurements of the TA muscle, the posterior cricoarytenoid muscle, and the diaphragmatic muscle as indicators of post-stimulation laryngeal muscle response, laryngospasm, and apnea. In the present study, the TA muscle was used as the primary indicator, but cricothyroid muscle contraction was also checked, inasmuch as it also reflects the magnitude and duration of laryngeal muscle contraction and is a useful extralaryngeal indicator of the state of the endolaryngeal musculature during laryngospasm.[38] The TA and cricothyroid muscles showed similar contraction patterns in our study; for example, neither indicated the presence of laryngospasm in the 2-week-old group, but laryngospasm was noted in some of the 4-week-olds and in even more of the 6-week-olds. That laryngospasm was more easily induced in the 4- and 6-week age groups than in the 2-week-olds suggests that the LCR is an age-dependent response that is absent in puppies younger than 2 weeks and appears after that age.

In general, the laryngeal reflex, including laryngospasm, is a protective reflex activated under the all-or-none rule, and an incremental increase in stimulation intensity does not induce any remarkable changes in the LCR response.[22] Other studies[7] have found that cessation of the laryngeal adductor reflex response was only achieved with the removal of the stimulating substance, but in this study, the laryngospasm or strong laryngeal contraction terminated earlier, or else initial laryngospasm and later strong contraction (biphasic contraction pattern or type 2 contraction) were noted before the removal of the stimulants. Hence, it must be considered that the depth of anesthesia might have been unstable or that a hyperoxic state might have been induced by overadministration of oxygen, or that there is immaturity of the central nervous system or nerves related to the laryngeal reflex arc in puppies less than 6 weeks of age. Given the latter possibility, it must be considered that fatigue aspiration may take place in the feeding of human infants or that laryngospasm may not occur during bronchoscopy in children less than 4 weeks of age.[37]

The respiratory transducer attached to the abdominal wall of each dog indicated a respiration pattern involving apnea or moderate respiratory suppression in the 4- and 6-week-olds. This result may be related to any or all of the facts that the rapid growth period of laryngeal function is between 4 and 6 weeks of age,[37] that the laryngeal adductor reflex is most hypersensitive in 7- to 11-week-old puppies,[12] and that severe central apnea has been noted in piglets less than 3 weeks of age.[4] After stimulation of the laryngeal mucosa with acid, respiration underwent an initial depression eventually followed by an elevation of respiratory rate. This compensatory respiratory increase may have occurred in response to blood gas changes, such as decreased arterial oxygen tension, which stimulated the peripheral arterial chemoreceptors.[7]

Some investigators have reported that the hemodynamic changes of the LCR response are hypertension and bradycardia,[4,28] but this finding differs from those of others, who noted hypotension and bradycardia,[23] hypotension and tachycardia,[33] or initial hypotension and bradycardia but later rebound hypertension and tachycardia.[7] One report[35] concluded that the baroreceptor mechanism may be related to a decline in heart rate, while another noted that the stimulation of the water receptors in the larynx did not directly affect blood pressure or heart rate, but that blood gas changes such as reduced oxygen tension during apnea might induce changes in the cardiovascular system, in which carotid body chemoreceptors may be involved in the control of blood pressure and heart rate responses.[32] In our study, heart rate did decrease after acid stimulation, but not to a statistically meaningful degree.

There have been many studies of the LCR response in a variety of experimental animals. The current study was based on the report of Loughlin et al,[34] which found an LCR response, including laryngospasm, after stimulation with strong acid solution at less than pH 2.5 in adult dogs. In the present study, differing LCR responses were detected in the 3 groups of puppies within 6 weeks of age, and these results may to some extent inform future research into sudden infant death syndrome.

CONCLUSIONS

The results of our LCR experiment in puppies were as follows.

1. Normal saline solution, distilled water, or cow's milk had no influence on respiration, heart rate, or laryngeal muscle contraction. However, strong acid solution (pH 1.0) induced an LCR response.

2. After stimulation of the laryngeal mucosa with acid solution, there was generally a depression of respiration initially, followed by a later elevation of respiratory rate. We found strong contraction or laryngospasm of the TA muscle after a short period of latency.

3. Respiration was not depressed, or was minimally depressed, in group 1 (2 weeks old). Moderate depression or apnea was elicited in groups 2 (4 weeks old) and 3 (6 weeks old). The average durations of apnea were 8.1 ± 1.4 seconds in group 2 and 9.2 ± 1.1 seconds in group 3.

4. After stimulation of the laryngeal mucosa with acid solution, the heart rate was reduced, but not to a statistically meaningful degree.

5. We found 3 types of TA muscle contraction pattern. Type 1 showed no laryngospasm, but some strong contraction was noted via EMG study. Type 2 showed no laryngospasm, but strong contraction was noted initially and was followed by some large waves on the EMG study. Type 3 showed laryngospasm that was visible to the naked eye and detected by EMG study. Laryngospasm was not seen in the 2-week-old group, but it was noted in 1 subject in the 4-week-old group and in 8 subjects in the 6-week-old group.

6. Peak-to-peak amplitude differences of TA muscle contraction were increased in all age groups; the increase was statistically significant in the 4- and 6-week-old groups.

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CHEILITIS GRANULOMATOSA MIESCHER: TREATMENT WITH CLOFAZIMINE AND REVIEW OF THE LITERATURE

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Cheilitis granulomatosa Miescher is a rare condition of unknown cause characterized by intermittent lip swelling that gradually persists and causes cosmetic deformity. We report the case of a young woman with cheilitis granulomatosa as a monosymptomatic manifestation of Melkersson-Rosenthal syndrome successfully treated by the antileprosy agent clofazimine, and propose clofazimine as an alternative treatment in cases refractory to corticosteroids. The differential diagnosis and current methods of treatment are summarized, and the literature is reviewed and discussed.

KEY WORDS — cheilitis granulomatosa, clofazimine, Melkersson-Rosenthal syndrome, Miescher's cheilitis.

INTRODUCTION

Cheilitis granulomatosa is a chronic inflammatory disorder of unknown cause. Originally described by Miescher[1] in 1945, this entity is clinically characterized by intermittent swelling of one or both lips that gradually persists. More recently, cheilitis granulomatosa has been considered to be part of the Melkersson-Rosenthal syndrome; now it is often discussed as a monosymptomatic form of Melkersson-Rosenthal syndrome.[2-4]

The common appearance of intermittent one-sided facial palsy and perioral swelling was first described in 1928, by the Swedish neurologist Ernst Melkersson.[5] A triad was established in 1931, by the neurologist Curt Rosenthal,[6] who added an associated lingua plicata. However, the full picture of the illness appears only in a small percentage of the patients.[3] Monosymptomatic and oligosymptomatic courses are no rarity.

The cause of this syndrome remains unclear. Bacterial or viral infections, allergic reactions, and vasomotor disturbances of the autonomous nervous system have been discussed as being possible causes, but have not been proven. As 61% of the patients have a possible family history, genetic inheritance could also be a factor.[2,3,7,8] The condition may start at any age, but shows a peak in the second decade and has an equal sex incidence. The condition follows a relentless course of fluctuant swelling, progressing to distortion of the normal anatomic architecture of the face. Spontaneous recovery may occur, but is rare.[3]

Findings in the morphological substratum are either characterized by epithelioid cellular granulomas or can show more inflammatory elements such as edema, lymphohistiocytic infiltrate, and fibrosis. As the cause of cheilitis granulomatosa is still unclear, the treatment is mostly symptomatic, anti-inflammatory, and/or antiproliferative. Steroids have been administered both systemically and intralesionally, but they have been of limited value.[9,10]

In 1986, Podmore and Burrows[11] reported 3 cases of histologically proven Miescher's granulomatous cheilitis and 1 case of Melkersson-Rosenthal syndrome treated successfully with clofazimine. In 7 patients with Melkersson-Rosenthal syndrome treated with clofazimine, beneficial effects were reported by Neuhofer and Fritsch.[12]

Clofazimine is a rhimophenazine dye originally developed for the treatment of tuberculosis. This drug has both antimicrobial and anti-inflammatory activities, and has found diverse use in the treatment of Hansen's disease (leprosy),[13,14] discoid lupus erythematosus,[15] and pyoderma gangrenosum[16,17] and in patients with human immunodeficiency viral infections who are also infected with *Mycobacterium avium* complex.[13]

Clofazimine is taken up by the reticuloendothelial system and fatty tissue on prolonged administration and may produce a reddish brown pigmentation that increases with ultraviolet exposure.[11] It affects the function of neutrophils, lymphocytes, and macrophages by mechanisms that are presently unknown.[18]

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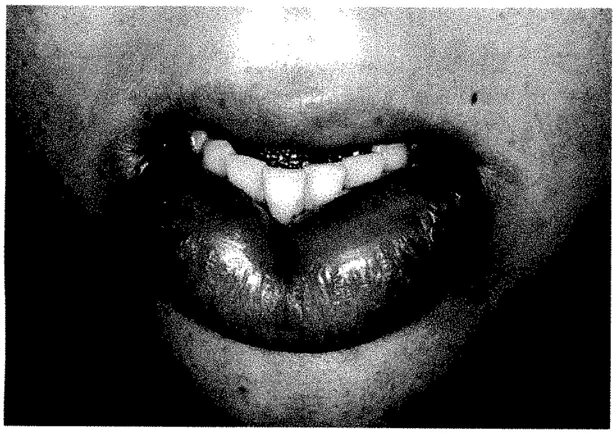

Distinctive homogeneous swelling of total lower lip without epithelium defect.

We report our experience in treating Miescher's granulomatous cheilitis with clofazimine in a 15-year-old patient in whom long-term steroid therapy was not practicable.

CASE REPORT

A 15-year-old girl was referred to the otorhinolaryngology department of the University Hospital Freiburg for a recurrent swelling of the left side of the upper lip that appeared 2 or 3 times a week during the night. She consulted her family physician who recommended astemizol initially. Her situation improved, but 3 weeks later, the left side of the lower lip swelled, and this time the swelling did not disappear. The patient complained about a cosmetic, very disturbing lip swelling, a feeling of tension, and paresthesias of the lower lip.

On clinical examination, the patient showed a soft, palpable swelling of the entire lower lip (see Figure). No other pathological findings could be found in the physical examination including intraoral and gingival examination. No facial palsy was noted. The laboratory tests, including serology studies for lues, hemolytic complement 50 (CH50), C1 esterase inhibitor, and latex antistreptolysin, yielded normal findings. Bacterial and fungal studies were negative. The allergy studies showed an IgE level of 2.4 kU/L without specific IgE antibodies. The patient was treated by oral methylprednisolone 40 mg without any improvement.

The lesion was submitted to biopsy, and the histologic examination showed that the entire submucous connective tissue was filled by small and moderate-sized granulomas that consisted of histiocytes, epithelioid cells, and giant cells. Near the vessels, infiltrations of plasma cells and lymphocytes could be found. The histologic findings were consistent with cheilitis granulomatosa.

The patient was treated with local injections of triamcinolone acetonide for a duration of 4 weeks, but no improvement occurred. After 6 months, a tentative treatment of a dosage of 100 mg clofazimine daily was initiated. After 30 days of treatment, regression of the swelling could be noted. Thereafter, 100 mg of clofazimine 3 times a week was given for 3 months. Under this treatment, the lip showed a significant reduction in swelling and eventually returned to normal size. No side effects were observed. The patient was followed up for a period of 6 years without recurrence of the disease.

DISCUSSION

Miescher's granulomatous cheilitis is a rare condition of unclear cause that is now considered a monosymptomatic feature of Melkersson-Rosenthal syndrome. Clinically, there is a swelling of one or both of the lips, usually the upper lip, which is initially fluctuant. This swelling progresses to a marked deformation of the lips with fissures on the surface and a reddish discoloration.[11] Other areas of the face may also swell (cheek, lower lip, nose, eyelids). Cheilitis granulomatosa causes distinctive lip swelling and cosmetic deformity, even to the point of considerable impairment of the quality of life for the patient. The clinical presentation ranges from a young age to early adulthood and is often monosymptomatic.[2,3]

In the differential diagnosis, hereditary angioneurotic edema, erysipelas, herpes recidivans, sarcoidosis, contact dermatitis, tumors (sarcoma), organized hematoma, and chronic infective granulomas should be taken into consideration. Granulomatous cheilitis has also been described as an early symptom of Crohn's disease.[19,20]

Histologically, granulomatous changes are seen around the vessels in the lamina propria with an inflammatory infiltrate that may be nonspecific or consist of giant cells with noncaseating epithelioid granulomas.[21]

Since the cause of cheilitis granulomatosa is still unclear, the therapy is now symptomatic, anti-inflammatory, or antiproliferative. Glucocorticosteroids are usually recommended initially, but only high doses or intralesional injections may be effective. In the literature, some case reports mention the use of metronidazole or a combination of prednisone and minocycline.[22,23] Surgical intervention to reduce macrocheilitis has also been recommended, but the success has been limited, and recurrences have been observed.[10]

Podmore and Burrows[11] described 3 patients with Miescher's granulomatous cheilitis and 1 with Melkersson-Rosenthal syndrome who were treated with clofazimine, including a 16-year-old boy. All 4 patients responded with clearance of granulomas demonstrated histologically after 4 months of treatment. The swelling cleared completely in 2 patients and partially, with some fluctuation of symptoms, in the other 2 patients.

Sussman et al[24] used clofazimine in 10 patients, including 1 child. Clinical improvement was reported in 8 of the adult patients; no results were available for the 12-year-old child.

Mahler et al[25] described a 59-year-old man who had granulomatous glossitis as an unusual manifestation of Melkersson-Rosenthal syndrome and was treated with clofazimine. His symptoms disappeared within 2 weeks, and recurrences were suppressed with a reduced maintenance dosage of clofazimine.[25]

Recently, we reported a case of laryngeal sarcoidosis refractory to steroids and successfully treated with clofazimine.[26] In this case and in other reported cases, no side effects of the treatment were noted.[7,11,27]

In 18 patients with Melkersson-Rosenthal syndrome, Tausch and Sonnichsen[7] used clofazimine for therapy. They found a decrease in the frequency and intensity of edema in 94% of patients, but after a follow-up period of up to 3 years, persisting improvement was seen in only 62% of patients.[7]

The mechanism of action of clofazimine is not fully known, but it is thought to be related to its ability to stimulate phagocytosis.[11,12,18,28,29] The distinctive lipophil affinity of clofazimine leads to an enrichment of the substance in the fatty tissue, in cells of the reticuloendothelial system, and in inflammable tissues. The removal of clofazimine takes place mainly over the biliary ducts, the kidneys, perspiration, sebum, and lacrimations. Very rarely, reversible increases of the blood glucose, the total bilirubin, and the transaminases can appear, as well as intestinal colic, diarrhea, anorexia, and nausea.[18,30] Clofazimine does not cross the blood-brain barrier, but does cross the placenta, and is found in human breast milk.[14] The elimination half-time is variable, with values as long as 70 days being quoted in the literature.

Clofazimine is a relatively safe drug if used in short courses and in low dosages, with the only side effect being a red discoloration of skin. There have been some reports of eosinophilic enteritis, but only after use of 600 mg of clofazimine daily for a 3-year period in leprosy patients in India.[28,31]

Because clofazimine can be given at an effective daily dose of 100 mg for a time period of up to 6 months with low toxicity and few side effects, it is of particular advantage. Further treatment cycles are possible at any time after an interval of approximately 3 months.[7] In approximately 20% of treated patients, a copper color to the skin can be seen, caused by oxidation in the lipid-rich tissues of the enriched phenazine derivative. The change in color of the skin is well tolerated by the patients. For prophylaxis of this effect, light exposure should be avoided during the period of treatment. Usually, after treatment is stopped, this side effect disappears.

Our patient responded well to treatment with clofazimime and was well and without a recurrence of the disease after 6 years of follow-up. On the basis of the reports in the literature and of the successful treatment with clofazimine in our case, we would suggest clofazimine as an alternative treatment for Miescher's granulomatous cheilitis in cases refractory to steroids.

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DYNAMIC ASSESSMENT OF PARANASAL SINUS VENTILATION USING XENON-ENHANCED COMPUTED TOMOGRAPHY

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Disease of the paranasal sinuses is a common and costly condition. Evaluation of the efficacy of either medical or surgical methods of treatment is limited by the lack of quantitative methods to characterize sinus ventilation, which may be an important determinant of the baseline physiological state of the sinuses. Xenon-enhanced computed tomography (Xe-CT) measurement of sinus ventilation provides a noninvasive method of quantifying maxillary sinus ventilation using the nonradioactive, radiodense gas Xe as a tracer. Study subjects breathed a mixture of Xe gas and oxygen through a close-fitting nasal mask during serial CT imaging of a single radiographic plane through the maxillary sinuses — a generally well-tolerated protocol. Analysis of the sinus density–time curves allowed calculation of first-order exponential time constants from which specific ventilation rates could be determined for individual sinuses. Previously developed data analysis techniques were used to assess the statistical significance of the data and determine confidence intervals, allowing examination of the effects of noise in the data, and to demonstrate areas for further study protocol refinement. We conclude that Xe-CT measurement of sinus ventilation is a potentially valuable noninvasive technique for the diagnostic imaging of the human maxillary sinus.

KEY WORDS — airflow, diagnostics, imaging, sinus, xenon ventilation.

INTRODUCTION

Disease of the paranasal sinuses is one of the most prevalent conditions seen by medical practitioners in the United States today, affecting as many as 30 million Americans. The annual cost of treatment of both chronic and acute sinus inflammation, excluding surgery, has been estimated at \$2.7 billion, and with surgical therapies, the cost may be as high as \$6.1 billion.[1,2] These enormous costs make sinus disease a major public health issue. The diagnosis of sinus disease is typically made by clinical history in conjunction with static imaging of the sinus cavities and nasal passages by x-ray and/or computed tomography (CT). Major therapeutic methods range from functional endoscopic surgery techniques to pharmacological intervention with steroids and topical decongestants and both long- and short-term antibiotic therapy. At present, however, there is limited ability to define the efficacy of either the pharmacological or surgical treatments apart from subjective relief of symptoms. For example, there remain groups of patients with significant clinical disability but apparently normal CT scan findings, as well as patients with abnormal CT scan findings that do not correlate with their symptoms. It has been suspected clinically by rhinologists that patients who have recurring nasal and sinus infections may have poor gas exchange in 1 or more sinuses. In addition, patients who seem not to improve after medical or surgical therapy may have too much or too little ventilation of their sinus spaces. In spite of a number of earlier investigations, there are no quantitative, in vivo, noninvasive methods for assessing the ventilatory function of the human paranasal sinuses at the present time. Establishment and validation of a technique for the dynamic assessment of airflow into and out of the sinuses, correlated with anatomic and symptomatic changes, would thus be of great clinical and research significance.

In his early discussions on the physiology of the nose, Proetz estimated that it would require several hours (more than 1,000 respirations) to completely exchange the air in the sinuses.[3,4] In 1977, Drettner and Aust,[5] using techniques based on oxygen (O2) content and exchange as measured with a polarographic O2 electrode placed in the sinuses, estimated an average exchange time of 5 minutes for air in the maxillary sinuses. In 1979, Zippel and Streckenbach[6]

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injected a mixture of ^{133}xenon and air into the frontal sinuses of normal humans via a cannula inserted through Beck's drill holes and measured the washout rate of the isotope as an indicator of gas exchange. They reported time-activity curves, well described by a monoexponential function, with times for gas exchange ranging from 1 to 10 minutes. The procedure was repeated for the maxillary sinus, with results comparable to those reported by Drettner and Aust, and the exchange times were found to be sensitive to changes in ostial patency and applied pressure. These early studies did establish that ventilation of the sinuses could be estimated by use of a tracer washout technique, but protocols involving drilling through the bony walls of the sinuses are obviously not suitable for routine clinical use.

The use of xenon-enhanced computed tomography (Xe-CT), in which the nonradioactive, radiodense gas xenon (Xe) is used as a contrast agent for CT scanning, was established in the late 1970s for the noninvasive measurement of cerebral blood flow[7,8] and pulmonary ventilation.[9,10] Briefly, stable Xe gas is denser than air, and its concentration may be measured by CT and is linearly related to its CT enhancement. Repeat CT scans taken at a fixed location during the wash-in or washout of a mixture of air and Xe yield exponential local density curves for a chosen region of interest (ROI). The time constant τ of the curve, determined from a nonlinear curve-fitting procedure, is equal to the inverse of the local ventilation per unit volume (specific ventilation). Thus, this technique provides a direct, noninvasive measurement of regional ventilation.

This technique was applied to the measurement of sinus ventilation by Kalender et al in 1985.[3,11] A group of 17 patients had balloon-tipped catheters placed in each nostril and underwent positive pressure insufflation of stable Xe into their sinuses during intermittent apnea over 5 minutes. The sinuses were then imaged by serial CT scans during normal breathing, and the washout rate of Xe was calculated from the changes in radiodensity. These authors found mean washout time constants τ (smaller τ means faster gas exchange) ranging from 2.6 minutes in the maxillary sinuses to 3.2, 4.9, and 14.6 minutes in the posterior ethmoid, sphenoid, and frontal sinuses, respectively.

Although there has been progress in the clinical use of Xe-CT for measuring cerebral blood flow and pulmonary ventilation, taking advantage of improvements in the speed and sensitivity of CT imaging, there have been no further reports of Xe-CT in the area of sinus study and characterization. We describe here an updated method for the noninvasive study of the paranasal sinuses in humans by the Xe-CT technique and present preliminary results from normal volunteer subjects along with a discussion of some of the strengths and limitations of the method.

MATERIALS AND METHODS

Subject Evaluation. Approval was obtained from the Institutional Review Board of Johns Hopkins Hospital. Written consent was obtained for each subject before CT scanning. Before the imaging study, a basic history and physical examination was obtained for each subject, including a review of medications, a review of sinus and head and neck history, a pregnancy test if applicable, and a standardized rhinosinusitis outcome measure questionnaire.[12] The subject was instructed to be without food or liquid intake (NPO) for a period of 8 hours before presenting for the Xe-CT study.

Subject Positioning. The subject remained in street clothes. After preliminary positioning in the CT scanner in the supine position, a wedge was positioned under the subject's knees. A snug-fitting nasal continuous positive airway pressure mask (Respironics Inc, Murrysville, Pennsylvania) was placed on the volunteer's head and secured with soft rubber straps. The head positioning approximated the reverse coronal position, allowing for imaging of anatomic planes containing the maxillary and sphenoid sinuses. The head position was then secured and maintained by molding and activating a vacuum-stabilized head cushion (Olympic Vac Pac, Olympia, Oregon). The arms were lightly secured across the chest with Velcro straps.

Subject Monitoring. Because Xe is a potent inhaled anesthetic, a Board-certified anesthesiologist was present during the entire protocol. The NPO requirement was strictly enforced, because inhaled Xe is sometimes associated with nausea and/or vomiting. The subject was monitored with continuous pulse oximetry. Supplemental face mask oxygen, suction equipment, intravenous fluids, and resuscitative equipment and drugs were on standby for all subjects. A remote television camera was positioned so that the subject could be closely viewed for signs of ventilatory distress or airway obstruction while in the CT scanner.

CT Specifications and Preliminary Images. Imaging was performed on a Siemens Somatom 4 CT scanner at 120 kV, 130 mA, and 3-mm slice thickness. After positioning, a lateral scout film was obtained. Next, 3 to 5 parallel test cuts through the maxillary and posterior ethmoid sinuses were obtained (Fig 1). A target plane was then selected that provided a clear view of the center of the sinuses and was flanked by at least 1 clear image on each side in order to mini-

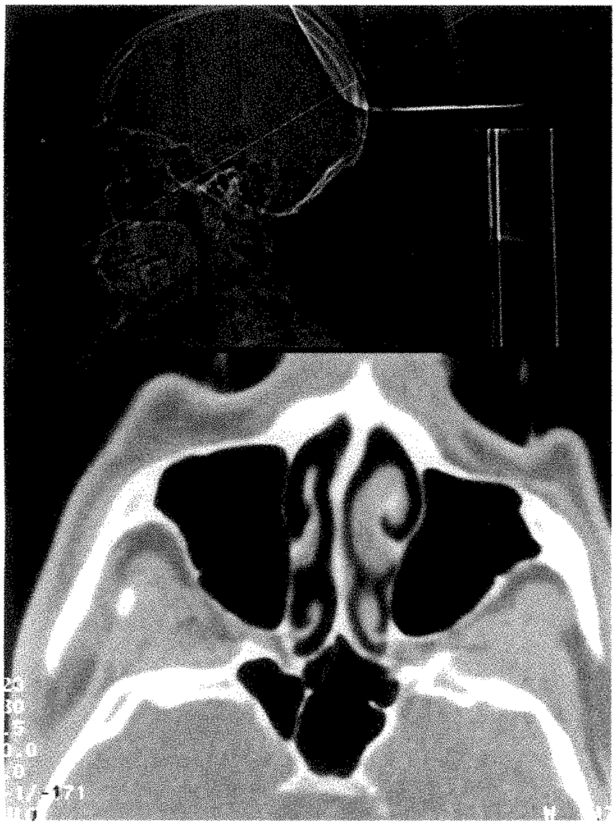

Fig 1. Lateral scout film with parallel test cuts and example computed tomography image plane through maxillary sinuses.

mize the risk of partial volume artifact from the sinus walls with subject movement.

Xenon-Oxygen Delivery. A 30% Xe–40% O2 mixture in air was delivered from the reservoir of an Enhancer 3000 system (Diversified Diagnostic Products, Houston, Texas), a commercially available device that mixes Xe and O2 in a closed circuit designed for Xe-CT cerebral blood flow studies. Inhaled and exhaled concentrations of Xe and carbon dioxide (CO2) were monitored and recorded on a 2-channel Gould strip chart recorder to ensure adequate ventilation and confirm Xe delivery. The subject was connected to the Enhancer system with a standard anesthesia breathing circuit, and an in-line heat and moisture exchanger was used to minimize any effects of cold, dry gas on the nasal physiology.

Xenon-Oxygen Wash-in–Washout Protocol. We focused this preliminary investigation on the ventilation of the maxillary sinuses. On the basis of our experience with Xe-CT pulmonary ventilation,[13,14] we used a wash-in–washout (wi/wo) protocol that maximizes the information obtained for a given radiation and Xe exposure. A total of 16 CT scans were performed during a 30-minute wi/wo protocol (Fig 2). Eight CT scans were performed during each half of the wi/wo protocol, with the subject breathing continuously via quiet nasal tidal respirations during both wash-in and washout of Xe. Image acquisition was not synchronized with the phase of respiration, and the images were obtained at predetermined times, with more frequent imaging during the early portion of each phase. After completion of the initial wi/wo protocol, the subject received topical nasal decongestion with spray (oxymetazoline hydrochloride 0.05%) and the protocol was repeated.

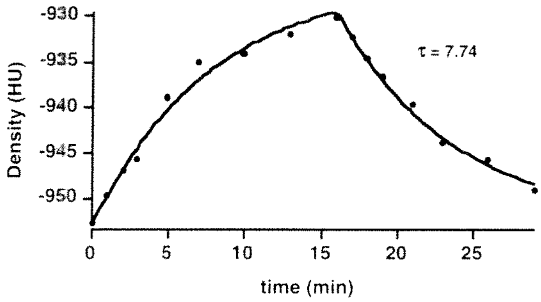

Fig 2. Example maxillary sinus wash-in–washout density data with first-order exponential fitted curve and time constant τ. Images are obtained at 0, 1, 2, 3, 5, 7, 10, 13, 16, 17, 18, 19, 21, 23, 26, and 29 minutes. Inhaled xenon is turned on at time 0 and off at 15 minutes 30 seconds. HU — Hounsfield units.

Data Analysis. Xenon wi/wo curves were obtained by measuring the density of an ROI defined within each maxillary sinus on the serial CT images. These time-density curves were then fitted to determine the first-order exponential time constant, τ, which was equal to the inverse of the specific ventilation (ventilation per unit volume) for that particular ROI within the cavity. The images were analyzed on a desktop computer using NIH Image, a public domain software package (available on the Internet at http://rsb.info.nih.gov/nih-image/). An ROI was defined for each of the maxillary sinuses by determining density thresholds to separate the sinus cavity from the bony walls. The sequential changes in radiodensity within this ROI, measured in Hounsfield units (HU), were then determined. A nonlinear curve fitting program (Igor Pro, Wavemetics, Lake Oswego, Oregon) was used to fit the density data to a first-order exponential model and determine the value of the time constant τ (Fig 2). The τ value was constrained to be the same for both the wash-in and washout portions of the curve. The 95% confidence intervals (CIs) were calculated for each value of τ with a previously described Monte Carlo simulation procedure.[14]

Protocol Follow-up. Because of possible residual anesthetic effects, subjects were required to be escorted home by a responsible party and were instructed not to drive or perform complicated tasks for the balance of the evening. Each subject was surveyed immediately after the completion of the wi/wo protocols and on the following day by telephone

SIDE EFFECTS

Subject	*Dizziness*	*Tingling*	*N/V*	*Altered Sensorium*	*Lightheadedness*	*"Rush"*	*Late Effects*
1	Mild	Yes	No	No	Mild	No	No
2	Mild	No	No	No	Mild	Yes	Sleepiness
3	No	No	No	No	No	Mild	No
4	No	No	No	No	Mild	Yes	No
5	Yes	No	Late	Yes	Yes	Yes	N/V
6	No	Yes	Yes	No	Yes	Yes	Nosebleed
7	No	No	No	No	No	No	No
8	No	Yes	No	Yes	No	Yes	No
9	No	Yes	No	No	Yes	No	No
Total positive	3/9	4/9	2/9	2/9	6/9	6/9	3/9

N/V — nausea and/or vomiting.

or e-mail. The subjects were asked to list and characterize the side effects of breathing Xe, their comfort level during the protocol, their willingness to recommend study participation to potential volunteers, and their willingness to undergo this protocol or a similar protocol if it were available to them as an indicated diagnostic procedure.

RESULTS AND DISCUSSION

There are 2 different areas in which the methodological issues and problems of evaluating sinus ventilation must be addressed, each equally important. First are the practical issues. The ease of undergoing this type of testing and the comfort and safety of the volunteers will ultimately factor highly in the development and use of the study protocol as a basis for a clinical diagnostic procedure. Because previous methods involved techniques that were difficult for the investigators, as well as procedures that were uncomfortable and invasive for the subjects, we have examined feedback from our subjects and considered the ease of administering the protocol. The second important area that must be examined in reviewing this method of sinus analysis as a viable investigational protocol involves establishing and evaluating the assumptions used in the analysis of the data and defining the ability of the protocol and the data analysis methods to adequately quantify, characterize, and define sinus ventilation over a range of normal values.

Overall, we find that the protocol is well tolerated in terms of both positioning and overall patient comfort. All subjects indicated an acceptable level of comfort with both the nasal mask and the positioning in the CT scanner. The reverse coronal head position is a standard radiologic imaging plane and generally can be maintained over the period of time during which the CT images are obtained. The vacuum head support will not absolutely prevent the subject from moving in the event of respiratory distress or emesis, but it provides enough stabilization so that very little motion artifact is seen on the CT scans.

The side effect profile experienced by our subjects after inhalation of 30% Xe–40% O2 in air is within the range described for this inhalation anesthetic.[15,16] For 7 of 9 volunteers, the effects of Xe inhalation were transient and mild, causing little or no distress (see Table). The study was terminated for 2 volunteers. One termination was at the discretion of the investigator, after completion of the initial wi/wo ventilation but before nasal decongestion was given, because the subject exhibited mild disorientation and dizziness. (About 1 hour later, he experienced nausea and 1 episode of emesis.) The second termination was for nausea and vomiting during the Xe washout phase of the second (after nasal decongestion) study. The subject later disclosed an unreported violation of the NPO requirement. Neither of these volunteers experienced respiratory compromise or distress of any kind. Both of these subjects reported an intolerance of amusement park rides and previous episodes of carsickness. This history might be used as a preprotocol screening criterion for prophylactic treatment with an antiemetic.

In any evaluation of study methods, the potentially hazardous exposures to which volunteers or patients are subjected are of paramount importance. This protocol was designed to minimize exposure to contrast medium and radiation exposure as compared to earlier techniques. Kalender et al[11] chose to insufflate pure Xe into the nasal cavity and sinuses during apnea in order to minimize breathing of the gas and to achieve as high as possible a concentration in the sinuses for improved imaging. Since that time, improved CT technology with faster acquisition and increased detector sensitivity and resolution allows the use of lower, subanesthetic concentrations with equal or better results. The use of 30% Xe is currently approved by the US Food and Drug Administration for Xe-CT cerebral blood flow studies. Thus, Xe-O2 mixtures may be breathed continuously via a nasal mask while imaging is performed, and awkward and inva-

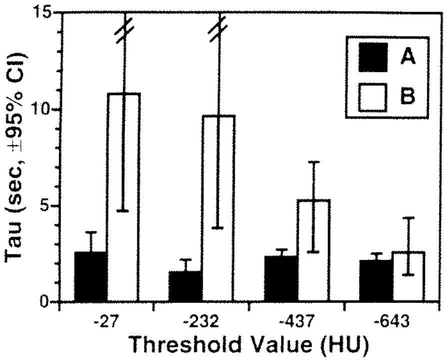

Fig 3. Time constants τ ± 95% confidence interval (CI) versus region of interest threshold values for 2 subjects. Curves for subject A had little noise, and there was little effect of changing threshold level. In contrast, data for subject B were very noisy at high threshold values (more dense tissue included), but excluding presumably intrasinus mucus and soft tissue by thresholding at −643 HU reduced noise and improved CI to acceptable level.

sive insufflation procedures can be avoided. Nonetheless, Xe gas is an inhalational anesthetic — about 30% more potent than nitrous oxide — and even at these low concentrations, side effects occur. One area of continued investigation with this protocol will involve establishing the lowest concentration of Xe that can be used to achieve valid characterization of sinus ventilation.

Radiation exposures were also minimized through careful choice of the timing of the CT scans (Fig 2). Image acquisition was planned such that adequate data are acquired for a range of τ values or specific ventilation rates by taking CT scans at shorter intervals during the periods when Xe is first being introduced or eliminated from the breathing circuit. Relatively fewer CT scans are needed during periods when there is a slower rate of change of concentration of Xe in the sinuses. The use of nonsynchronized imaging with respect to the ventilatory cycle was based on preliminary studies in which no differences in the wi/wo curves or τ values were noted between end-inspiratory and unsynchronized imaging protocols, and nonsynchronized studies are easier for the subject to undergo.

It is estimated that the total radiation dose to the skin surface of each subject as a result of the 2 sets (before and after decongestion) of CT scans will be 23 mSv (2.3 rem). This is equivalent to an effective dose or uniform total body dose of 0.4 mSV (40 mrem). This radiation exposure is well below the level of 300 mrem, the radiation exposure received by the average individual in the United States from natural background radiation sources, which is one of the primary guidelines used by the institutional review board of Johns Hopkins Hospital in determining the safety of human studies involving radiation.

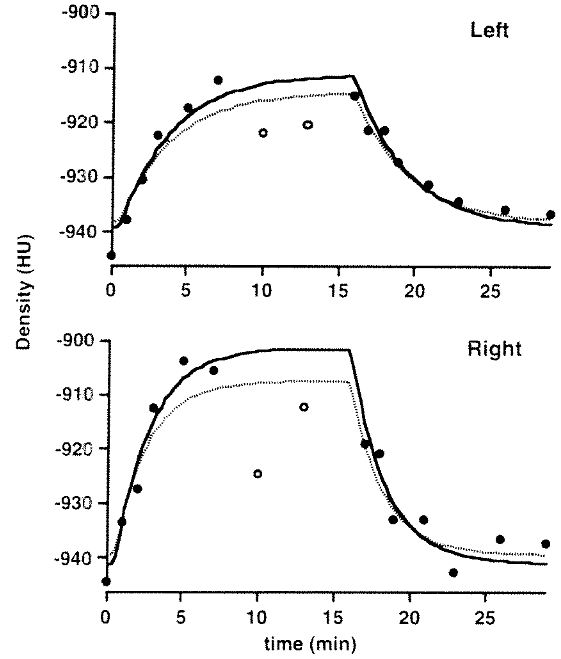

Fig 4. Effect of mouth breathing on maxillary sinus wash-in–washout curves. Eliminating data points in which xenon concentration was diluted by breathing room air (open circles) significantly changes fitted curves (dashed to solid lines), time constants, and confidence intervals (see text for details).

Data Analysis. In order to determine the ROI in each image, we used a threshold function to separate the sinus cavity from the surrounding bone and tissue on the basis of density. The specific value of this threshold parameter can have a significant influence on the time constant and noise level of the Xe-CT wi/wo curve by determining what is included in the sinus ROI. The ideal ROI would include all of the sinus air space while excluding surrounding bone, soft tissue, and mucus. Thresholding allows the maximum sinus area to be included in each image, selected by means of a uniform criterion, and helps to reduce the noise and partial volume effect errors introduced through head movement and physiological cyclical changes in sinus mucosal thickness. To determine an appropriate threshold value, we first visually compared the ROI boundaries obtained by thresholding images over a range of values from −27 to −643 HU. Next, the wi/wo curves from a fixed ROI chosen in the center of the maxillary sinus (well away from the walls) were compared to those obtained from the same CT images thresholded at different values. The contributions of different levels of thresholding to noise in the wi/wo data set were assessed by evaluating the standard deviations and CIs of the corresponding values for τ. For CT images in which there is not a significant amount of low-density interference from the presence of mucus and soft tissue in the sinus, the effect of thresholding may be minimal

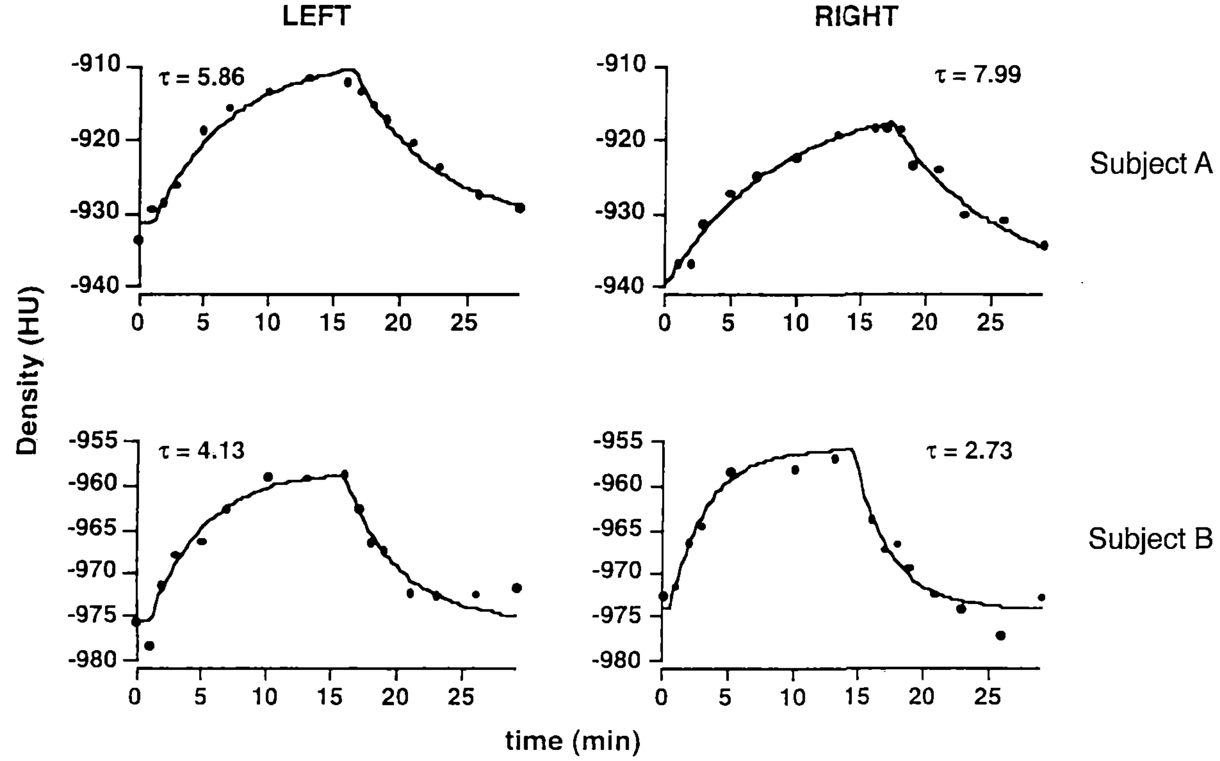

Fig 5. Fitted maxillary sinus wash-in–washout curves from 2 subjects. Ninety-five percent confidence intervals determined by Monte Carlo analysis reveal that ventilation time constants of left and right sinuses of subject A are statistically significantly different, whereas those of subject B are not.

(Fig 3, subject A). For Xe wi/wo data sets with greater noise, the threshold value may have significant impact on the calculated τ value (Fig 3, subject B) and, more important, the CI. Optimal ROIs are determined by thresholding images within the range of −443 to −643 HU to define the tissue-air boundary.

An additional source of error in the wi/wo data is the subject's respiring by mouth during the scanning protocol. Since room air contains no Xe, mouth breathing dilutes the Xe in the nasal passages and sinuses and lowers the sinus radiodensity. Typically, this occurs during the middle portion of the wi/wo protocol, and it is perhaps due to the mild sedating effect of the inhaled Xe. An example of the effect may be seen in the wi/wo curves of a subject who respired by mouth for an interval at the end of the wash-in phase of a protocol (Fig 4). This mouth breathing was identified during the protocol by a decrease in the end-expired Xe concentration noted on the digital display of the Enhancer. The subject successfully resumed nasal ventilation after prompting. Excluding these diluted points as artifact may have a substantial impact on the curve fitting and statistical analysis of the sinus ventilation rates. The uncorrected τ values (95% CI range) for this subject for the left and right sinuses were 3.03 (1.71 to 5.11) and 1.8 (0.57 to 3.85) seconds, respectively. The τ values changed to 3.02 (2.9 to 4.03) and 1.97 (1.41 to 2.55) seconds after these 2 data points were eliminated from the curve fitting. For this example, the corrected τ values were not statistically different from the uncorrected, but the width of the 95% CI was reduced by 40% to 60%, so that the ability of the study to detect changes in sinus ventilation was greatly improved. Of course, one must exercise caution in eliminating any data points from analysis. To reduce the possibility of including a confounding error in the analysis, we continuously record end-tidal CO_2 and Xe concentrations on a strip chart recorder, so that intervals of mouth breathing may be more readily identified.

Although the Xe-CT wi/wo method described here can quantitate sinus ventilation in terms of the wash-in time constant τ, the interpretation of these values is greatly enhanced by the additional determination of the 95% CI about this calculated τ value. The CIs were calculated by a Monte Carlo simulation method[14] that estimates the uncertainty in the value of τ that results from the noise in the data. This powerful technique allows the determination of a CI about each τ value, without requiring repeated measurements that would mean duplicating the entire Xe-CT study several times and greatly increasing radiation and Xe exposure. Knowing the CI permits statements to be made as to the statistical meaning of comparisons between individual measurements. For example, in

1 subject, τ values of 5.86 and 7.99 were calculated for the left and right maxillary sinuses, respectively (Fig 5, subject A). The lack of overlap of the 95% CI for these values (4.90 to 6.78 versus 6.91 to 9.33) suggests that these values are statistically different. For a different subject, there is considerable noise evident in the data (Fig 5, subject B). The τ values for the left and right sinuses are 4.13 and 2.73, respectively. Although the absolute difference between these values is smaller than in the prior example, examination of the 95% CIs (3.10 to 5.38 versus 2.24 to 3.49) shows that this difference is not statistically significant. An important application of the Monte Carlo error analysis will be the interpretation of changes in sinus ventilation that occur with interventions such as nasal decongestion.

Another application of these Monte Carlo techniques is to allow theoretical comparisons of different experimental protocols. For example, it is possible to compare the effects of using subsets of the entire wi/wo data set on τ, and the CI about τ, in order to determine the trade-off between fewer data points (less radiation exposure) and more frequent imaging (improved curve fitting for determining τ). For example, the wi/wo data described above (Fig 5, subject A) were reanalyzed after a number of manipulations were done to the data: elimination of every fourth data point, every third data point, and every other data point. Eliminating up to every third data point did not cause a statistically significant change in either the value of τ for either sinus (compared to all data points included) or the difference between the two sinuses. However, eliminating every other data point caused the CI ranges to increase to the point that the ability to detect a difference between the sinuses was lost. Using simulations such as this, based on real data, will allow further optimization of the imaging protocol to minimize radiation and Xe exposure as discussed above.

With the present protocol and radiation exposure levels, determination of the specific ventilation of only the maxillary sinuses has been possible. Although the sphenoid sinus is well visualized in all of the CT images, no consistent density enhancement has been measurable. This could be because there is a prohibitive signal-to-noise ratio due to interference from bony structures, or simply because much longer wi/wo times are needed to transfer enough of the tracer Xe into this sinus. The ethmoid and frontal sinuses are not visualized in the radiologic plane used in this study. Although the size of the frontal sinus varies greatly in humans, for individuals with average-size or larger frontal sinuses it is anticipated that an imaging plane can be located that would permit analysis by the Xe-CT method. The ethmoid sinuses present the greatest difficulty for this technique, both because of their small size and because of the number of bony partitions inherent in sinuses with a large number of air cells. Further studies using different imaging planes and longer imaging protocols will help determine the optimal application of this technique for studying all of the sinuses.

CONCLUSIONS

We conclude that our protocol for dynamic Xe-CT imaging using a subanesthetic mixture of O_2 and Xe respired by nasal continuous positive airway pressure mask is a noninvasive, generally well-tolerated technique with which to characterize and investigate the dynamic ventilation of human maxillary sinuses in the clinical setting. Studies that characterize the range of sinus ventilation values in normal subjects and in patients with sinus disease will determine the utility of this technique as a diagnostic tool.

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SCREENING OF OLFACTORY FUNCTION WITH A FOUR-MINUTE ODOR IDENTIFICATION TEST: RELIABILITY, NORMATIVE DATA, AND INVESTIGATIONS IN PATIENTS WITH OLFACTORY LOSS

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The study aimed to create a screening test on the basis of the odor identification test as used in the "Sniffin' Sticks" olfactory test kit. It should appeal to the practitioner in terms of 1) time required for testing, 2) reliability, 3) separation of "normal" from "abnormal," and 4) the fact that it allows lateralized screening. Experiments should provide a normative database (number of subjects > 1,000), establish test-retest reliability (n > 100), and compare results from patients with olfactory loss (n > 200). The correlation between results on 2 repetitive tests was .78. The test differentiated anosmics, hyposmics, and normosmics ($p < .001$). None of the 112 anosmics reached a score higher than 8; the 90th percentile was at a score of 6. When only 6 odors were used for calculating scores, for anosmics the 95th percentile was at a score of 4. These data provide a basis for the screening of patients by means of "Sniffin' Sticks."

KEY WORDS — anosmia, odor discrimination, odor identification, odor threshold, olfaction.

INTRODUCTION

"Sniffin' Sticks" is a test of nasal chemosensory function based on felt-tip pens that was devised by G. Kobal in Erlangen, Germany. In its most elaborate version, it comprises 3 tests of olfactory function, namely, tests for odor threshold, odor discrimination, and odor identification.[1,2] This test provides detailed information on different olfactory functions. However, its application takes approximately 20 to 30 minutes. Thus, because of the time involved in its application, it is mostly used in specialized centers.[2,3] The practitioner is often left with quick but unstructured tests of olfactory function, many of which provide little information.[4]

To remedy this situation, we previously introduced an 8-item olfactory screening test.[5] However, as the test at the time of its construction had to be tailored according to the guidelines of the German Society for Otorhinolaryngology–Head and Neck Surgery, it made use of 3 "pure" odorants, namely, phenyl ethyl alcohol ("rose" odor), alpha-ionone (flowery odor), and limonene ("citrus" odor). The other items included 3 "mixed, trigeminal-olfactory" stimulants (eucalyptol, eugenol or "cloves," and iso amyl acetate or "solvent"), a strong trigeminal stimulant (mustard oil or "mustard"), and an odorless blank. Because of these limitations, the test was shown — despite its test-retest reliability of $r_{24} = .73$ in healthy subjects, and promising initial results in patients with olfactory loss[5] — to have a poor resolution in the discrimination between anosmia and normosmia.[4,6] In fact, empirically it was shown that some anosmics correctly identified as many as 7 items.[6]

Consequently, initiated by the Working Group on Olfaction and Gustation of the German Society for Otorhinolaryngology–Head and Neck Surgery, the aim of the present study was to create a screening test on the basis of the "Sniffin' Sticks" odor identification subtest. The result should appeal to the practitioner in terms of 1) time needed for application, 2) reliability, and 3) separation of "normal" from "abnormal." Finally, 4) it should be possible to perform the screening in a lateralized fashion.

To address these questions, we performed the following 3 experiments. Test-retest reliability was established in more than 100 healthy subjects, results from more than 1,000 subjects were used to create a normative database related to age, and finally, we compared results from more than 250 patients in whom normosmia, hyposmia, or anosmia had been determined by extensive psychophysical and electrophysiological olfactory testing.

MATERIAL AND METHODS

In all experiments described below, we used a 16-item odor identification test based upon the "Sniffin'

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Sticks" test kit.[1] As a rule, both sides of the nose were tested in each subject. In cases in which lateralized testing was performed, the results for the better nostril were used for further statistical evaluation. This approach was adopted on the assumption that the better nostril largely determined the results obtained during bilateral measurements.[3,7]

Psychophysical testing with the "Sniffin' Sticks" involves the presentation of odorants in felt-tip pens. The pens have a length of approximately 14 cm and an inner diameter of 1.3 cm. The pen's tampon is filled with dissolved odorants. For odor presentation, the cap is removed by the experimenter for approximately 3 seconds and the pen's tip is placed approximately 2 cm in front of both nostrils. Odor identification was assessed by means of 16 common odors. On a multiple-forced-choice task, identification of individual odorants was performed from a list of 4 descriptors each. The interval between odor presentations was approximately 20 seconds. The subjects' scores ranged from 0 to 16. The odorants were handled extremely carefully to prevent olfactory contamination. The measurements were performed in quiet, well-ventilated rooms.

In the first part of the study, data were obtained from 1,012 healthy (normosmic) subjects (490 female, 522 male; median age, 40 years; age range, 5 to 86 years) evaluated at the outpatient clinic of the Department of Otorhinolaryngology of the University of Dresden Medical School, Dresden, Germany. None of these consecutive patients had major nasal disorders or presented because of olfactory disorders. In this part of the study, we tried to address the question of to what degree the 16 odors were identified by healthy subjects. In addition, these data should provide the normative database for later clinical applications of the olfactory screening test.

The second portion of the study was performed on 112 anosmic patients (57 female, 55 male; median age, 57 years; age range, 13 to 89 years), 103 hyposmic patients (57 female, 46 male; median age, 51 years; age range, 12 to 84 years), and 40 normosmic subjects (20 female, 20 male; median age, 49 years; age range, 29 to 83 years). All subjects were thoroughly investigated; ie, they underwent a detailed physical examination by an otorhinolaryngologist, their olfactory function was tested by means of the "Sniffin' Sticks" test battery including tests for odor discrimination and odor thresholds, electrophysiological recordings were made in order to verify the presence of anosmia, and a detailed history was taken.

The diagnosis "anosmia" was based on the patient's report of olfactory loss and a "Sniffin' Sticks" test score of less than 16.[2] In addition, anosmia was verified by the absence of olfactory-evoked potentials in response to stimulation with 2 ppm hydrogen sulfide.[8-10] Electrophysiological recordings were made by a technique previously described by Kobal.[11] Odor stimuli were embedded into a constant flow of air to prevent concomitant mechanical or thermal stimulation (stimulus duration, 200 ms; total flow, 8 L/min; 80% relative humidity; 35°C to 37°C). Responses were recorded from the scalp at midline positions Fz, Cz, or Pz (referenced to A1/A2), and vertical eye movements were monitored at the Fp2 lead. The electroencephalogram (EEG) was digitized at a rate of 250 Hz. For averaging, 8 to 20 single records of 2 seconds' duration were available. During testing, white noise was used to mask clicks associated with stimulus presentation. The diagnosis "hyposmia" was based on the patient's reports and a "Sniffin' Sticks" test score of less than 31.[2]

In the third portion of the study, we investigated the test-retest reliability of the 12-item identification test (interval between test and retest, 1 to 55 days). To this end, we reanalyzed data that had been obtained previously in healthy subjects.[1] This data set included 105 subjects (52 female, 53 male; mean age, 49.5 years; SD, 18.5; age range, 18 to 84 years). In addition, we included data from 8 consecutive patients with hyposmia (4 female, 4 male; mean age, 45.8 years; SD, 16.1; age range, 22 to 78 years). Criteria for inclusion of these patients were 1) a maximum interval between test and retest of 6 months, 2) a duration of olfactory dysfunction at the time of the first test of more than 1 year, and 3) no nasal surgery during the interval between tests.

Finally, the data were analyzed with regard to a possible lateralized application. First, 2 groups of 6 odor pens each were created. The identification rates were not different between the groups of odor pens (group 1, 84.8% mean correct identification of items; group 2, 85.4%; *t*-test, $p = .63$). Group 1 comprised the following odors: banana, licorice, fish, cinnamon, rose, and peppermint; group 2 consisted of lemon, pineapple, leather, coffee, cloves, and orange. We investigated 1) how anosmics scored on the lateralized test, 2) how good the test-retest reliability was, and 3) how large the differences between left and right nostrils were in normosmic subjects.

The results were analyzed by means of SPSS 9.0 for Windows. Because olfactory sensitivity varies as a function of age,[12,13] the subjects were separated into age groups of 10 years' width. To explore olfactory sensitivity in relation to age and gender, data were submitted to analyses of variance (ANOVAs) using the general linear model and multifactorial

TABLE 1. PERCENTAGES OF CORRECT IDENTIFICATIONS (1 OF 4 ITEMS, MULTIPLE FORCED CHOICE) BASED ON RATINGS OF 1,012 SUBJECTS

Odor	*Percent Correctly Identified*
Garlic	46.2
Turpentine	50.2
Apple	54.6
Cinnamon	72.6
Banana	78.8
Lemon	79.3
Anise	80.9
Licorice	81.3
Pineapple	81.7
Coffee	84.6
Cloves	85.4
Rose	87.5
Leather	89.7
Fish	90.3
Orange	90.5
Peppermint	93.6

design ANOVAs with "between-subject factors," "group," and "gender" with Bonferroni post hoc tests. The differences between results obtained for the left and the right nostrils were analyzed by means of an ANOVA for repeated measures (general linear model, within-subject factor "nostril"). For investigations of differences between normosmics, hyposmics, and anosmics, test scores were investigated in relation to both gender and cause of disease with an ANOVA (general linear model, multifactorial design; between-subject factors "gender" and "cause"). The alpha level was 5%. Correlational analyses were performed with Pearson statistics. All investigations were performed according to the Declaration of Helsinki (Summerset West Amendment).

RESULTS

Selection of Odors. The percentages of correct odor identifications in the 1,012 healthy subjects in the first portion of the study are shown in Table 1. Among the 16 odors used, garlic, turpentine, and apple were identified at rates below 55%; hence, they were left out of the olfactory screening test. All other odors were identified by more than 70% of the investigated subjects. In addition to the 3 odors mentioned above, "anise" was excluded from the set of odors used in the screening test, because the odors "anise" and "licorice" of the 16-item odor identification test were regarded as too similar to each other to be represented in the screening test. Thus, the screening test was based on 12 items.

Effects of Age and Gender. When the 1,012 healthy subjects were binned in 9 groups according to the decades of their ages (Table 2), we found a significant decrease of odor identification ability with increasing age (for factor "age," F[8,994] = 15.58 and $p < .001$). The odor identification scores were lowest for the oldest subjects investigated (Fig 1). For the effects of gender, no significant difference was found (for factor "gender," F[1,994] = 2.08 and p = .15), although on average, female subjects had higher identification scores than did male subjects.

Normative Data. Descriptive statistics of the 1,012 healthy subjects are presented in Table 2, separately for 9 age groups.

Differentiation Among Anosmic, Hyposmic, and Normosmic Subjects. For the 255 subjects given precise diagnoses by means of extended testing with both the "Sniffin' Sticks" and olfactory evoked potentials, the screening test was clearly able to differentiate

TABLE 2. DESCRIPTIVE STATISTICS OF ODOR IDENTIFICATION SCORES OBTAINED IN 1,012 HEALTHY SUBJECTS, LISTED SEPARATELY FOR 9 AGE GROUPS

		Age (y)								
		<11	*11-20*	*21-30*	*31-40*	*41-50*	*51-60*	*61-70*	*71-80*	*81-90*
No. of subjects		33	148	159	175	139	158	132	59	9
Mean		9.6	10.6	10.8	10.6	10.5	9.9	9.5	8.3	8.1
SEM		0.3	0.1	0.1	0.1	0.1	0.2	0.2	0.3	0.6
Median		10.0	11.0	11.0	11.0	11.0	11.0	10.0	9.0	8.0
SD		1.5	1.6	1.5	1.8	1.6	2.3	2.5	2.6	1.9
Minimum		6.0	3.0	3.0	1.0	4.0	1.0	1.0	1.0	5.0
Maximum		11.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	11.0
Percentile	10	7.4	8.0	9.0	9.0	9.0	6.0	5.3	4.0	5.0
	20	8.0	10.0	10.0	10.0	10.0	9.0	8.0	6.0	6.0
	30	9.0	10.0	11.0	10.0	10.0	10.0	9.0	7.0	7.0
	40	10.0	11.0	11.0	11.0	11.0	10.0	10.0	8.0	8.0
	50	10.0	11.0	11.0	11.0	11.0	11.0	10.0	9.0	8.0
	60	10.0	11.0	11.0	11.0	11.0	11.0	11.0	10.0	9.0
	70	10.8	12.0	12.0	12.0	11.0	11.0	11.0	10.0	9.0
	80	11.0	12.0	12.0	12.0	12.0	12.0	11.0	11.0	10.0
	90	11.0	12.0	12.0	12.0	12.0	12.0	12.0	11.0	11.0

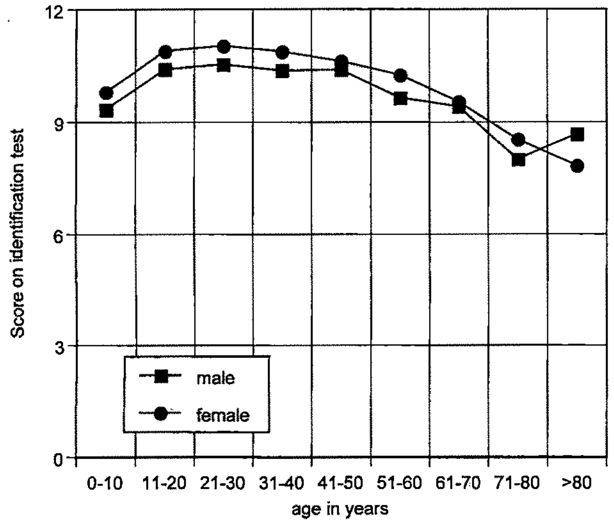

Fig 1. Age-related changes of odor identification scores in healthy subjects (N = 1,012; means), shown separately for male and female subjects in 9 decades (0 to 10 years, N = 33; 11 to 20 years, N = 148; 21 to 30 years, N = 159; 31 to 40 years, N = 175; 41 to 50 years, N = 139; 51 to 60 years, N = 158; 61 to 70 years, N = 132; 71 to 80 years, N = 59; 81 to 90 years, N = 9). With exception of youngest subjects, scores decreased as function of age. Although average scores were typically found to be higher in female subjects, factor "gender" did not reach level of significance.

group data from anosmics, hyposmics, and normosmics (F[2,254] = 199.32, p < .001; Table 3). Post-hoc Bonferroni tests also indicated that these differences were present for multiple comparisons among the 3 groups (p < .001). All anosmics had scores of 8 or lower (Fig 2). On the basis of the 90th percentile of the score distribution of anosmics, all patients with a score of 6 or lower can be considered to have a severe olfactory problem. On the basis of a 90th percentile test score of 10 in hyposmics, a score of 11 or 12 is indicative of normal olfactory function. Scores in the range from 7 to 10 may be indicative of hyposmia; however, they clearly do not exclude the presence of normosmia or, to a lesser degree, anosmia.

Test-Retest Reliability. The correlation between results on tests separated by a maximum of 6 months was r_{113} = .78 (Fig 3).

TABLE 3. DESCRIPTIVE STATISTICS OF ODOR IDENTIFICATION SCORES OBTAINED IN ANOSMIC, HYPOSMIC, AND NORMOSMIC SUBJECTS

		Anosmia	*Hyposmia*	*Normosmia*
N		112	103	40
Mean		3.7	6.6	10.3
SEM		0.2	0.2	0.2
Median		4.0	6.5	10.5
SD		1.7	2.2	1.3
Minimum		0.0	2.0	7.0
Maximum		8.0	12.0	12.0
Percentile	10	1.0	4.0	9.0
	50	4.0	6.5	10.5
	90	6.0	10.0	12.0

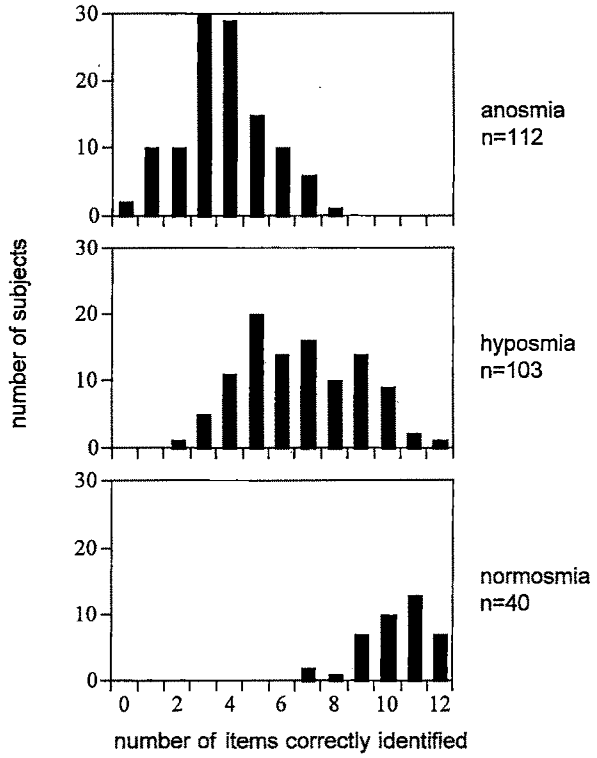

Fig 2. Distribution of scores on 12-item odor identification test, shown separately for anosmics (n = 112), hyposmics (n = 103), and normosmics (n = 40). Data from hyposmics show considerable overlap with data from anosmics and normosmics. Using 90th percentile of respective distributions for anosmia, cutoff of 6 could be considered; for hyposmia, 90th percentile is at 10.

Lateralized Testing. With regard to the use of 6 items each for lateralized testing, anosmics reached the maximum possible score of 6. However, in anosmia, the 90th percentile for the distribution of scores was a score of 3, and the 95th percentile was a score

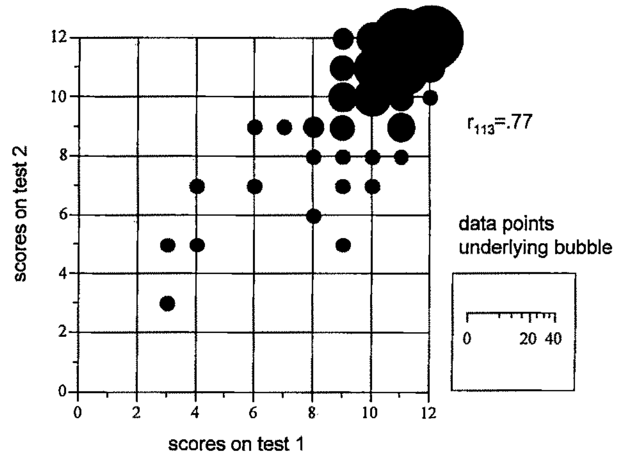

Fig 3. Test-retest reliability of 12-item odor identification test in 113 subjects. Coefficient of correlation (r_{113} = .77) indicates good correlation between repeated measurements.

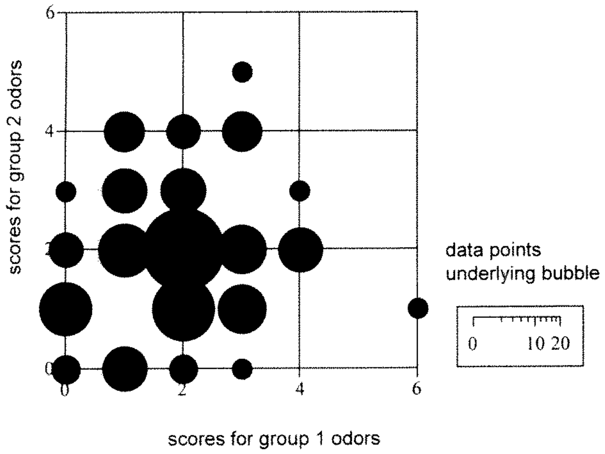

Fig 4. Scores attained by subjects with anosmia when 12-item odor identification test was divided into 2 groups of 6 odors each. Only 2 of 113 subjects (1.8%) reached score higher than 4.

of 4 (Fig 4). In hyposmia, the 90th percentile was 5, and the 95th percentile was 6, indicating considerable overlap between normosmics and hyposmics (Fig 5). When re-investigating the 113 subjects (see above), we observed test-retest reliabilities of r113 = .55 for group 1 odors and r113 = .76 for group 2 odors.

When reanalyzing the data from the sample of 1,012 healthy subjects, we found the differences between the data obtained for group 1 odors and those obtained for group 2 odors to be small. In the overwhelming majority of subjects (97.8%, n = 990), the differences between scores for the 2 groups of odors were 2 or less; in 1.9% (n = 19) of the subjects, the difference was 3; and in the other 0.3% (n = 3), the difference was 4 or higher.

DISCUSSION

Our studies provide a basis for the routine clinical use of this screening test. The test is extracted from a 16-item odor identification test with "Sniffin' Sticks."[1,2] The screening takes approximately 4 minutes to be administered. It has a test-retest reliability of r113 = .77, which is similar to that of other tests that use similar numbers of items.[14] It screens for olfactory dysfunction, and — with limitations — can even be applied in a lateralized fashion.

The anosmic subjects reached a maximum score of 8. The 90th percentile of the score distribution in the anosmics was 6, indicating that all patients with a score of 6 or lower have a high probability of having severe olfactory dysfunction. Correspondingly, the 90th percentile in subjects with hyposmia was 10. As there is considerable overlap between people with hyposmia and anosmia, and between people with hyposmia and normosmia, only scores of 11 or 12 may be indicative of normal olfactory function. To differentiate between hyposmia and normosmia, scores below 11 warrant a more detailed olfactory investigation, eg, the extended version of the "Sniffin' Sticks" test.[1,2]

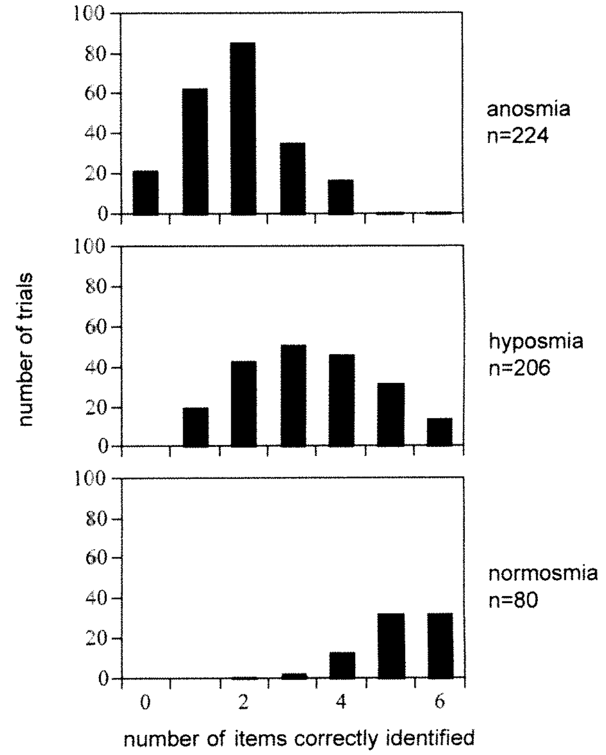

Fig 5. Scores attained by subjects with normosmia, hyposmia, and anosmia when 12-item odor identification test was divided into 2 groups of 6 odors each. In anosmia, 90th percentile for distribution of scores was at score of 3, and 95th percentile was at score of 4. In hyposmia, 90th percentile was at 5, and 95th percentile was at 6. These findings indicated that this lateralized 6-item test could only be used to screen for anosmia.

The present results also indicate that the 12-item test can be used for lateralized olfactory testing with 6 odors for each nostril. A cutoff score of 4 represents the 95th percentile of the distribution of scores in anosmics. Thus, in a clinical environment, a score of 4 or less would call for extended olfactory testing, whereas scores of 5 or 6 would indicate the absence of anosmia. Such information on left- and right-sided olfactory function would be extremely helpful in counseling patients with lateralized anosmia who are considering surgery that has the potential to endanger the remaining olfactory function.

Olfactory dysfunction is frequently seen in patients with neurologic disorders. Consequently, tests of olfactory function have been suggested in the differential diagnosis of Parkinson's disease[15-17] and Alzheimer's disease.[18-20] Many neurologists only question the patient with regard to his or her olfactory abilities. The quick odor screening test presented here might be of help in this situation.

Another important aspect of this test is pricing, which will be well below US $100 (Burghart Instruments, Wedel, Germany; oral communication). Considering a shelf life of approximately 1 year and the possibility of repeated use of this kit, the cost-benefit ratio of this validated tool appears to be satisfactory.

Scores obtained in healthy subjects clearly exhibited a decrease in olfactory function over time, forming the basis for specific normative values in relation to a subject's age. Age-related decreases in olfactory sensitivity have already been established in numerous previous studies.[21-24] The present data obtained in more than 1,000 subjects once more confirm reports of a decrease in subjects' ability to identify odors after the age of 60 years.[12]

Taken together, our results provide a basis for the routine clinical screening of olfactory dysfunction. Although the results of this test are clearly limited with regard to a precise estimate of olfactory sensitivity, they provide the practitioner with a gauge for olfactory abilities that can be applied in less than 4 minutes. Furthermore, the test can be administered such that it allows screening for the presence of lateralized anosmia.

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INVOLVEMENT OF LEVEL I NECK LYMPH NODES IN ADVANCED SQUAMOUS CARCINOMA OF THE LARYNX

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This study was performed to evaluate the incidence of metastasis at level I in patients with squamous laryngeal cancer. One hundred consecutive patients with squamous carcinoma of the larynx were submitted to surgical treatment including radical neck dissection. The tumor stage was T3 or T4, and the neck stage was N1-N2c. Lymph node metastases were pathologically confirmed in 80 patients. Metastases were concentrated within level II in 59% of cases, level III in 17% of cases, level IV in 11% of cases, and level V in 6% of cases. Only 2 patients (2%) had detectable tumors in the lymph nodes of the submandibular triangle (level IB). This study shows that patients with laryngeal cancer rarely present metastases at the submandibular triangle, even in advanced local disease with cervical metastasis staged as N1 to N2c. Therefore, dissection of the submandibular triangle is indicated only in the presence of clinical, radiographic, or cytologic evidence of metastatic disease at level I.

KEY WORDS — laryngeal neoplasm, lymph node, metastasis, neck dissection, surgery.

INTRODUCTION

Squamous cell carcinoma of the head and neck accounts for approximately 6% of all new cancers diagnosed in the United States yearly. Of these malignancies, 25% arise on the larynx. The incidence of lymph node metastases varies from 25% to 65%,[1-3] and the presence and extension of these metastases are significant prognostic factors.[4-6] Despite the variety of operations designed to remove cervical lymphatics, which ranges from the classic radical neck dissection described by Crile[7] to the modifications of Suárez[8] and Byers,[9] any of these procedures is ideal in offering maximal cure while preserving appearance and function in patients with clinically metastatic lymph nodes. The lymphatic drainage of the larynx occurs along predictable pathways, and level II, III, and IV lymph nodes are the most frequently involved. Metastases in level I or level V are rare and are usually associated with metastases in lymph nodes along the internal jugular vein.[2-4,10,11]

We conducted this retrospective study to analyze the prevalence of level I lymph node metastases from primary laryngeal squamous cell carcinoma in a series of 100 patients, all of whom underwent classic or modified radical neck dissections.

PATIENTS AND METHODS

This report is based upon the retrospective analysis of 100 consecutive patients with previously untreated squamous cell carcinoma of the larynx who were treated from 1955 to 1997 in a single institution. All patients were initially submitted to a total or near-total laryngectomy and classic or modified radical neck dissection, followed by postoperative radiotherapy in 57 cases. With regard to gender, 88 patients were male and 12 were female, with ages ranging from 37 to 80 years (mean, 56.4 years).

All patients presented with clinically metastatic ipsilateral neck lymph nodes. On the basis of the clinical staging system proposed by the American Joint Committee on Cancer (AJCC)[12] and by the TNM Committee of the International Union Against Cancer (UICC),[13] the patients were restaged and classified so that 68 patients were considered to have T3 cancer and 32 patients were considered to have T4 cancer. The neck stages were N1 in 65 patients, N2a in 16 patients, N2b in 10 patients, and N2c in 9 patients.

The surgical treatment of the primary tumor was always associated with a simultaneous classic or modified radical neck dissection. Total laryngectomy was performed in 61 patients, total laryngectomy and partial pharyngectomy in 38 patients, and near-total laryngectomy in 1 patient. All patients underwent radical neck dissection: 13 type III modified radical neck

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N STAGE BY SITE OF POSITIVE NODES ACCORDING TO AJCC[12] AND UICC[13] 1997 CLASSIFICATION

Level Involved	*N Stage*				
	N1	*N2a*	*N2b*	*N2c*	*Total*
pN0	18	2	0	0	20
IB	0	1	1	0	2
II	35	8	10	6	59
III	7	3	4	3	17
IV	4	1	3	3	11
V	1	1	2	2	6
VI	0	0	2	2	4

dissection, 43 unilateral radical neck dissection, 27 bilateral radical neck dissection, and 17 radical neck dissection on the side of the primary tumor and simultaneous lateral neck dissection (levels II, III, and IV) of the contralateral side of the neck.

Postoperative radiotherapy was used in 57 patients. The interval from the time of surgery to the onset of radiotherapy ranged from 2 to 8 weeks. During the period of patient accrual, indications varied broadly because of the characteristics of the primary tumor (close or involved surgical margins and presence of vascular embolization or perineural infiltration) or the presence of regional lymph node metastases (independent of the number of involved lymph nodes, level of involvement, or transcapsular spread). The postoperative radiotherapy consisted of a wide-field technique including both sides of the neck with a dose range of 50 to 65 Gy. The radiation source was a linear accelerator of 4 MeV in 30 cases and a cobalt beam in 27 cases.

RESULTS

Neck metastases were diagnosed in the surgical specimens in 80 of the 100 patients initially submitted to unilateral or bilateral neck dissections. The number of pathologically positive nodes varied from 1 to 18. Ipsilateral metastases were found in 80 of the 100 patients, and contralateral metastases in 14. Metastatic disease was concentrated within the jugular chain (level II in 59% of patients, level III in 17%, and level IV in 11%). There were 6 patients with metastasis at level V. Only 2 patients had detectable tumors within the lymph nodes of the submandibular triangle (level IB; see Table[12,13]). None of the patients who had submandibular nodal metastasis presented recurrences in the neck during the follow-up.

Forty-two patients presented tumor recurrences (9 local, 15 neck, 12 distant, and 6 associated). None of the patients had recurrences at level I. A second primary tumor was diagnosed in 7 patients. Follow-up information was available for all of the patients. At the study closing date, 39 patients had died of cancer recurrence or treatment complications, 16 had died of non–cancer-related causes, and 31 were alive with recent follow-up information. Fourteen patients had been lost to follow-up.

DISCUSSION

The necessity of the inclusion of level I, which contains the submental (level IA) and the submandibular (level IB) triangles, in radical and modified neck dissections is questionable for the treatment of advanced cancer of the larynx and hypopharynx with neck metastasis.[14,15] The neck is anatomically divided into 7 nodal regions.[16] The submental triangle or space is bounded laterally by the anterior bellies of both digastric muscles, inferiorly by the hyoid bone, and superiorly by the symphysis of the mandible. The mylohyoid muscle forms the floor of this triangle. The submandibular triangle is designated as part of level I and is bounded anteriorly by the anterior belly of the digastric muscle, posteriorly by the posterior digastric belly, superiorly by the body of the mandible, inferiorly by the hyoid bone, superficially by the platysma, and deeply by the floor-of-mouth structures.

In a review of lymph node metastasis distribution in a large series of previously untreated head and neck malignancies, Lindberg[1] noted that the submandibular triangle was involved only when primary tumors were located in the oral cavity or anterior oropharynx. If the primary tumor was located in the hypopharynx or supraglottic larynx, the incidence of metastatic spread to the submandibular triangle was less than 1%.[1] These data have been corroborated by subsequent reports that used pathology data.[2,17]

Dissection of the submandibular triangle is usually relatively safe, but there are potential complications. Permanent unilateral or bilateral damage of the marginal mandibular and hypoglossal nerves has been reported.[18] The operative time is somewhat increased and the possibility of vascular injury and hemorrhage is heightened when the submandibular triangle is included in a neck dissection.[14]

Pietrantoni and Fior,[19] in a review of 570 cancers of the larynx and hypopharynx, stated that the submandibular triangle should not be included in neck dissection. Candela et al[2] found that level I was involved in 5% of clinically N0 necks, and only 7.8% of N-positive necks had nodal involvement at this level when the larynx was identified as the site of the primary tumor. Wenig and Applebaum[14] found only a 2% incidence of lymph node metastasis in the submandibular triangle in a series of 95 patients with cancer of the larynx and hypopharynx, and they concluded that in the absence of clinical or radiographic evidence of metastatic spread to the submandibular triangle, dissection of this area was not justified. Redaelli de Zinis et al[20] encountered only 1 case of lymph node involvement at level I in 99 patients with lymph node metastases from supraglottic squamous cell carcinoma, and in this case, also, other levels were involved. More recently, Nicolai et al[21] observed that in a series of 402 consecutive patients treated for supraglottic squamous cell carcinoma, level I nodes were involved only in 0.5%.

The data presented in this and other studies, although retrospective in nature, corroborate our impression that tumors of the larynx rarely spread to the submandibular triangle. Only 2% of the patients included in this series with advanced local disease had cervical metastasis staged as N1 and N2A. Another important consideration is that there is no impact of level I involvement on overall survival (data not shown), and the preservation of this area reduces the duration of the surgical procedure, as well as the risk of complications. There are strong arguments associated with the reduction of treatment cost, without jeopardizing the oncological radicality, and cosmetic and functional advantages. Therefore, dissection of the submandibular triangle should be performed only in the presence of significant clinical, radiographic, or cytologic evidence of metastatic disease at level I.[15] Dissection of the anterior aspect of level I, the submental triangle, is not indicated, as nodal involvement, which occurs rarely in the submandibular triangle in advanced cases, does not occur in the submental triangle.[22]

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CLINICAL NOTE

ATYPICAL FIBROXANTHOMA

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This is the first case report of atypical fibroxanthoma in the otolaryngology literature. Atypical fibroxanthoma is a rare fibrohistiocytic tumor that occurs on actinically damaged skin. The typical presentation is a nodular lesion in sun-exposed areas of the head and neck in an elderly patient. Differentiating atypical fibroxanthoma from other soft tissue sarcomas, such as dermatofibrosarcoma protuberans and malignant fibrous histiocytoma, can be difficult. Treatment should be wide local excision with a 1-cm margin. In cosmetically important regions in which tissue preservation is important, Mohs' surgery should be considered.

KEY WORDS — atypical fibroxanthoma, dermatofibrosarcoma protuberans, malignant fibrous histiocytoma, soft tissue sarcoma.

CASE REPORT

A 77-year-old woman presented with a 4-month history of a right auricular mass. The mass was a 2.5 × 3-cm, nodular lesion involving the antihelix and a portion of the concha (Fig 1). Biopsy revealed a spindle cell proliferation with pleomorphic nuclei and cellular atypia. On immunohistochemical examination, the lesion was negative for keratin, S-100 protein, and HMB-45, and positive for vimentin. A diagnosis of atypical fibroxanthoma (AF) was made. The patient underwent wide local excision of the lesion with reconstruction with a full-thickness skin graft. Histology again showed a spindle cell neoplasm. However, this time the lesion was called a dermatofibrosarcoma protuberans (DFSP). It should be noted that the wide local excision was done at a different facility, so the pathologist who read the second case was different from the one who read the initial biopsy. Within 1 month, the patient developed a swelling under the skin graft, suspicious for a recurrence. She was taken back to the operating room, where another wide local excision was done with frozen section control. The final margins were all negative. Underlying cartilage was excised and did not show involvement with tumor. The defect was left to heal by secondary intention. Histology again showed a spindle cell neoplasm. Two months later, the tumor recurred. At this time, the rapid recurrence, despite the frozen section control, suggested that the original neoplasm may not have been an AF, but rather, a more aggressive sarcoma. The fact that a diagnosis of DFSP was entertained after the first wide excision was also of concern. Consequently, the cell blocks from the biopsy and the 2 excisions were sent to the Mayo Clinic, Rochester, Minnesota, for further review. The Mayo Clinic supported the initial diagnosis of AF over DFSP on the basis of the fact that there was significant cellular atypia and nuclear pleomorphism and because the tumor was CD34-negative on immunohistochemical analysis (see below). It was not thought to be malignant fibrous histiocytoma (MFH), as there was no invasion of deeper tissues, namely, the auricular perichondrium and cartilage. The patient was referred for Mohs' surgery. The lesion was excised, and the first stage of reconstruction was done at the same sitting. The patient is disease-free 4 months after surgery, and the staged reconstruction is being completed.

DISCUSSION

Atypical fibroxanthoma is a fibrohistiocytic tumor. While it has been described previously by derma-

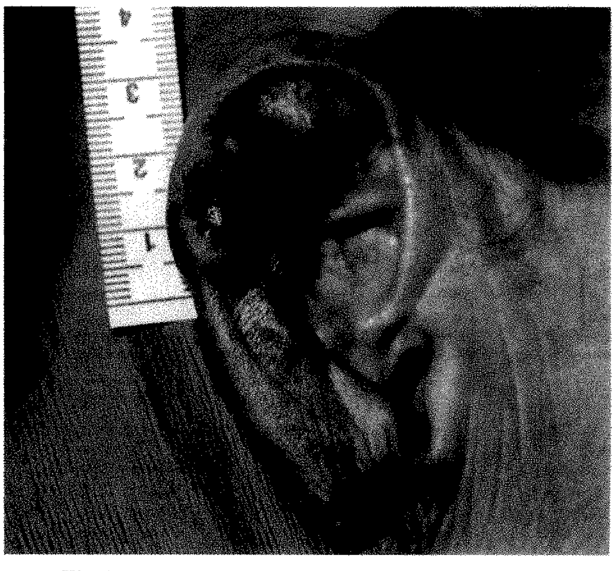

Fig 1. Right auricular mass on initial presentation.

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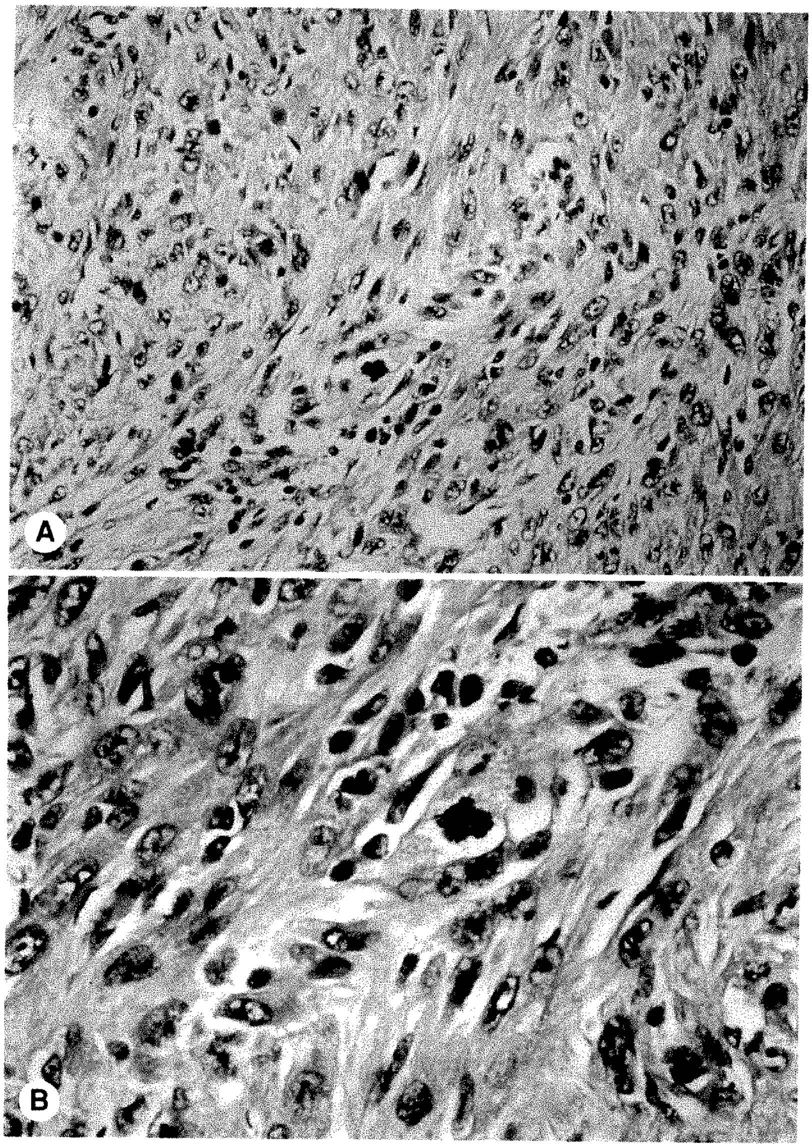

Fig 2. Atypical fibroxanthoma. **A**) Low-power view shows overall spindle cell architecture. **B**) High-power view shows bizarre mitotic figures, nuclear atypia, and cellular pleomorphism.

tologists, this the first case report of AF in the otolaryngology literature.[1-3] It is useful to think of AF, DFSP, and MFH as soft tissue sarcomas along a spectrum. Atypical fibroxanthoma is considered a superficial, low-grade sarcoma that, once excised, has a low recurrence rate. The present case illustrates that this is not always true. Dermatofibrosarcoma protuberans is a more aggressive neoplasm with deeper invasion and more tendency to recur. Malignant fibrous histiocytoma is even more aggressive, frequently recurs, often metastasizes, and has a poor overall prognosis.[2,3] The present case highlights how difficult it can be to distinguish among these lesions. The lesion was initially called AF, then DFSP, and finally, again, AF after extensive review.

The typical presentation of AF is as a nodular lesion on sun-exposed skin of the head and neck in an elderly individual.[1-4] The primary locations are the ears, nose, cheeks, and scalp. In contrast, DFSP and MFH tend to occur at different sites (see Table). Histologically, AF is a dense spindle cell neoplasm.[1-4] The cells show significant pleomorphism, mitotic activity, and nuclear atypia[1-4] (Fig 2). Atypical fibroxanthoma is, histologically, often indistinguishable from MFH, except that in AF there is no invasion of deeper subcutaneous tissue, fascia, or muscle. Thus, some authors consider AF to be "MFH in situ."[3] In contrast, DFSP has cells in a characteristic whorled pattern. There is minimal pleomorphism and mitotic activity.[3,4]

The differential diagnosis for these soft tissue sarcomas includes malignant melanoma and carcinoma.[2,3] Immunohistochemical analysis is important in distinguishing the soft tissue sarcomas from these other lesions, as well as in differentiating among AF, DFSP, and MFH. All 3 sarcomas are vimentin-positive, S-100 protein–negative, and HMB-45-negative. Dermatofibrosarcoma protuberans is CD34-positive,

TUMOR DATA

	Atypical Fibroxanthoma	*Dermatofibrosarcoma Protuberans*	*Malignant Fibrous Histiocytoma*
Typical sites	Mainly head and neck	Trunk, extremities; 10% head and neck	Extremities; 10% head and neck
Histology	Pleomorphism, atypia	Whorled pattern, less atypia, less pleomorphism	Like atypical fibroxanthoma, but invades deeper tissues
Immunohistochemistry*	CD34-negative	CD34-positive	CD34-negative
Surgical margins	1 cm	2 to 3 cm	5 cm
Recurrence rate†	10% to 20%	40% to 50%	40% to 50%

*All 3 are negative for S-100 protein, keratin, and HMB-45.

†Even with proposed margins.

whereas AF is CD34-negative.[3]

Wide local excision is the treatment of choice. Some authors have advocated at least a 1-cm margin for AF.[3] Others have questioned this recommendation, as AF is supposed to be a less-aggressive neoplasm. The present report suggests that AF can be a locally aggressive lesion and that the 1-cm guideline may be prudent. In contrast, DFSP and MFH are more invasive neoplasms, and wide margins, ranging from 3 to 5 cm, have been advocated[3] (see Table). Even then, high recurrence rates have been reported for DFSP and MFH. Consideration should be given to Mohs' surgery in an attempt to conserve as much tissue as possible.

The role of radiotherapy in treating AF is unclear. While some authors have demonstrated that fibrohistiocytic tumors respond to radiotherapy, others have raised concerns about whether radiotherapy could actually induce more malignant behavior.[1-3]

CONCLUSION

Atypical fibroxanthoma is a rare neoplasm that must be distinguished from DFSP and MFH. Treatment is surgical excision with at least a 1-cm margin. Consideration should be given to Mohs' surgery for recurrent lesions and, perhaps, even as the primary treatment method in areas in which a 1-cm margin would entail significant cosmetic or functional morbidity.

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IMAGING CASE STUDY OF THE MONTH

CHRONIC PETROUS APICITIS WITH PERICAROTID EXTENSION INTO THE NECK IN A CHILD

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INTRODUCTION

With the arrival of modern antibiotics, infectious processes of the petrous apex, called petrous apicitis or petrositis, have severely declined in frequency. Nevertheless, this complication of otitis media still occurs and carries major intracranial risks (meningitis, extradural abcess, and brain abscess). Moreover, as clinicians, we should remain alert, because the symptoms of petrous apicitis can be rather subtle, and today patients often present without the full triad known as Gradenigo's syndrome (abducens nerve paralysis, retro-orbital pain, and ipsilateral aural discharge).[1]

The spread of bone destruction to the petrous apex occurs by contiguity if the petrous apex is pneumatized, but can also proceed by direct extension, even in a sclerotic or diploic petrous apex. Once blood vessels with their surrounding adventitia are reached, infection can spread to distant areas.[1,2] We describe an extremely rare pediatric case with long-standing chronic middle ear infection and complicated by petrous bone destruction and spread of infection along the carotid canal and deep cervical fascia to the upper neck region. In the radiographic workup, computed tomography (CT) is important in the detection of osseous erosion, whereas magnetic resonance imaging (MRI) provides more information about the composition and extension of the lesion.[3,4] In order to guarantee functional integrity in this case, we combined the infralabyrinthine-retrofacial approach for drainage of the apex with a cervical exposure of the neck abscess.

CASE REPORT

A 9-year-old girl had been afflicted with recurrent episodes of otitis media since early childhood. At the age of 4, she was treated with adenoidectomy and insertion of grommets. After extrusion of the left tube, a small perforation remained. The ear had drained intermittently over the past 2 years. Repeated suction associated with ototopical, as well as general, antibiotic treatment did not resolve the infection definitively. She also presented, 1 year before she was seen by us, a left retroauricular fistula that disappeared after a few months with conservative management.

She was referred to us because of the sudden appearance of an infra-auricular swelling of about 4 cm. Micro-otoscopy revealed a small, wet, anterior perforation. Pure tone audiometry showed an average 30-dB conductive hearing loss. The neck lump located in the posterior cervical triangle was painless and was neither warm nor red.

A contrast-enhanced CT scan was performed to evaluate this posterior neck abnormality. A peripheral enhancing hypodense mass was found in the left posterior cervical triangle, in the suboccipital region (Fig 1A). There was an extension along the left carotid space to the skull base, in which an enhancing lytic mass of the left petrous bone apex was found.

Extension around the left internal carotid artery with invasion of Meckel's cavity was noted (Fig 1B). Because of its multiplanar imaging capabilities, MRI demonstrated to advantage the peculiar extension of the mass in the left petrous bone apex, along the carotid space and toward the component in the left posterior cervical triangle (Fig 2). Extension was also noted into the internal auditory canal, on the posterior aspect of the petrosal pyramid. There was no enhancement of the membranous labyrinth (Fig 3). The imaging characteristics of the petrous apex lesion were nonspecific. On T2-weighted images, the mass appeared slightly hyperintense. On T1-weighted images, the lesion appeared isointense to brain paren-

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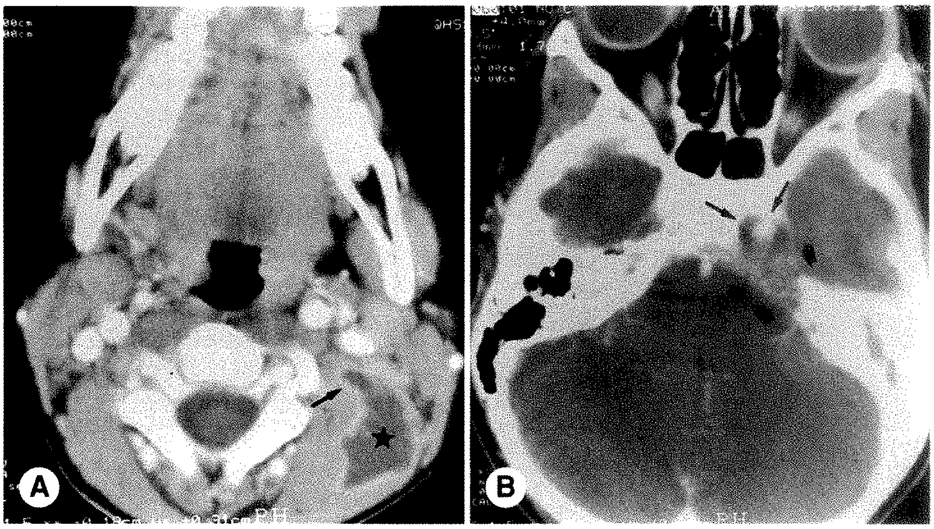

Fig 1. Axial contrast-enhanced computed tomograms. **A)** At level of upper jugular region. Shows hypodense mass with peripheral enhancement in left posterior cervical region (asterisk). Note small extension toward left carotid space (arrow). **B)** At level of skull base. Shows lytic mass with enhancement in left petrous bone apex (short arrows). Note extension around left carotid artery and invasion of Meckel's cavity (long arrows).

chyma. Strong enhancement was noted on the images after intravenous administration of gadolinium. The component along the left carotid space and in the left posterior cervical triangle demonstrated a central hypointensity with peripheral enhancement on the T1-weighted images after gadolinium was used. Diffuse enhancement of the middle ear and mastoid region was also found. On the basis of the lytic appearance of the petrous apex lesion and the associated "collection-like" component in the posterior cervical region, diagnosis of an aggressive infectious and/or inflammatory lesion was made.

An aural swab showed some scattered gram-positive cocci and bacilli. Aerobic, anaerobic, and fungal and yeast cultures were unproductive. Blood tests and serologic studies were not indicative of chronic inflammatory disease. Skin tests showed no delayed hypersensitivity for the most prevalent mycobacteria or for fungal pathogens. A chest X-ray was normal.

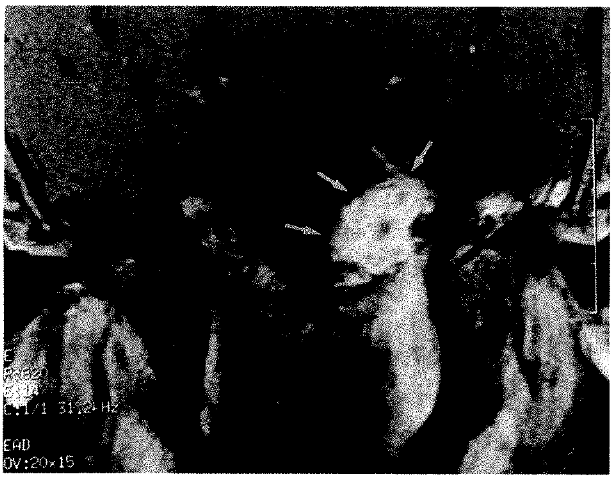

Fig 2. Coronal T1-weighted magnetic resonance image taken through skull base and brain stem after intravenous administration of gadolinium shows strongly enhancing mass in left petrous bone apex (white arrows). Coronal incidence clearly shows strongly enhancing extension along left carotid space (black arrows).

The patient underwent a transmastoid biopsy. The frozen section revealed an extensive necrotizing, granulomatous inflammatory reaction with no evidence of microorganisms. Further cleaning of the mastoid and tympanic cavity was performed during the same procedure with extensive removal of mucopolypoid and necrotic mucosa. Also, cell tracts in the supralabyrinthine, retrolabyrinthine, and infralabyrinthine directions were opened and cleaned. The petrous apex was finally drained through an infralabyrinthine retrofacial route. In order to obtain maximal exposure,

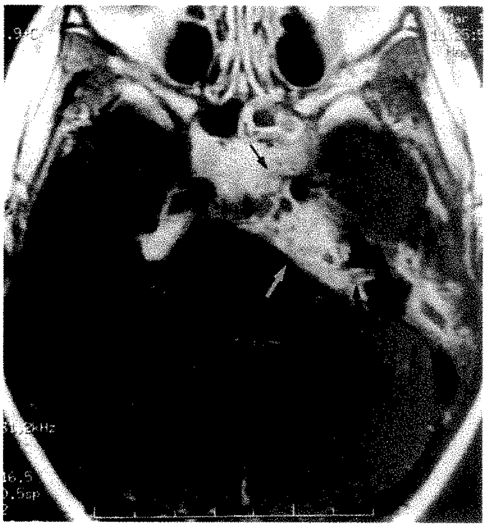

Fig 3. Axial T1-weighted magnetic resonance image taken at level of petrous bone (membranous labyrinth) after intravenous administration of gadolinium. On this image, large enhancing mass is found in left petrous bone apex (white arrow) with extension around left carotid artery and invasion of Meckel's cavity (short black arrows). Note invasion of left internal auditory canal (long black arrow) and enhancement of mastoid cavity. Compare with normal right side.

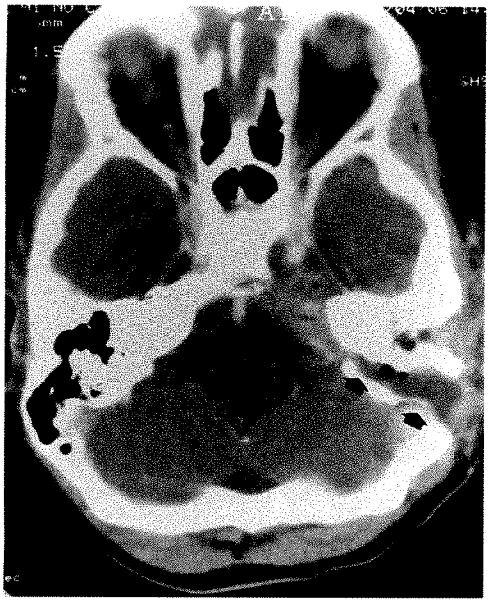

Fig 4. Axial enhanced computed tomogram taken at level of petrous bone. Infralabyrinthine approach is clearly shown (arrows).

we skeletonized the facial nerve and jugular bulb and carefully pushed the bulb down in order to gain sufficient access to the apex and the carotid canal. The apical cells were lined with thick mucopolypoid mucosa and were filled with granulation tissue in the midst of which caseous and necrotic matter was found, as well as bony sequestrae. Pressure applied on the upper neck region elicited an upward flow of pus along the carotid canal, toward the apical abscess. After complete cleaning of the apex, including the adventitia of the carotid artery, the retroauricular incision was extended downward into the neck. The cervical abscess was incised and drained behind the sternocleidomastoid muscle and flushed with an antibiotic solution (rifampicin). The perforated eardrum was then reconstructed, while the ossicular chain, still intact, required no reconstruction. For the first 5 postoperative days, repeated washouts were performed through the cervical and mastoid drains with the same antibiotic solution. The child recovered uneventfully, and the average hearing level improved from a preoperative level of 30 dB to 15 dB. The tympanic membrane remained stable during the 1-year follow-up period. Further pathological and bacteriologic studies on the operative specimens did not reveal any responsible pathogen.

On a postoperative contrast-enhanced CT scan, the infralabyrinthine approach can clearly be seen (Fig 4).

DISCUSSION

Although petrous apicitis was a frequent occurrence in the first half of the 20th century, it has become an uncommon disease because of the widespread use of antibiotics for otitis media.[1,2] In chronic otitis media, *Staphylococcus*, *Pseudomonas*, or other gram-negative organisms may become trapped at the apex and evolve into an abscess. In our case, the abscess was probably sterilized by the repeated treatments with local and systemic antibiotics. Apicitis usually presents as episodes of pain and persistent aural discharge, and anterior apicitis may be complicated by a sixth nerve paralysis.[2,3] In contrast with other cases, ours presented without the complete triad of symptoms known as Gradenigo's syndrome.

In general, the differential diagnosis of a petrous apex lesion is based on several criteria, such as the age of the patient, the aspect of the mass, and associated lysis on CT, as well as the signal intensities and contrast enhancement on MRI.[3,4] In the case of an aggressive-appearing lytic lesion in the petrous bone apex in a child, one should always keep infectious, inflammatory, and tumoral lesions in mind. All of these lesions can have nonspecific imaging characteristics on MRI and can demonstrate an aggressive and lytic appearance on CT.[5]

Although petrous apicitis classically may demonstrate abscess by a collection-like appearance on MRI with central low signal intensity on T1-weighted images, high signal intensity on T2-weighted images, and peripheral enhancement after intravenous administration of gadolinium, aspecific signal intensities with diffuse enhancement may frequently be found. On CT, pneumatization of the petrous bone apex can be found. These lesions should always be differentiated from tumoral lesions. Rhabdomyosarcoma is the most common head and neck malignancy in children. About 7% of rhabdomyosarcomas develop in the temporal bone. On CT, extensive indiscriminate bone destruction is found. On MRI, once again, signal intensities are nonspecific, with generally strong enhancement after use of gadolinium. Temporal bone Langerhans' cell histiocytosis can have similar nonspecific imaging characteristics. In this case, however, the differential diagnosis was substantially limited by the collection in the left posterior cervical triangle.[5] This case presented in an extremely rare manner, extending from the apex along the carotid artery and down into the neck, following the deep cervical fascia.

For drainage and debridement, the retrofacial infralabyrinthine route was preferred because of the smaller risk of meningitis as compared with the combined supralabyrinthine and middle fossa approach. The cervical abscess was separately drained through an inferior extension of the retroauricular incision.

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BOOK REVIEWS

Physiology of the Ear

Anthony F. Jahn and Joseph Santos-Sacchi, editors. Second edition. Hard cover, illustrated, indexed, 689 pages, 2001. Singular, San Diego, Calif, $89.95.

Purpose: To show, 13 years after the first edition, that which is relevant in the physiology of the ear regarding research and therapeutics.

Contents: There are 28 chapters that could be divided into physiology of the external ear, middle ear, and inner ear. The book is heavily weighted to the inner ear, with 18 chapters devoted to it, only 1 of which is on the physiology of the vestibular system. There are 40 contributors, 33 of whom have PhDs.

Attributes and Limitations: The essence of Georg von Békésy's elucidation of Helmholtz's theory of hearing was based on his observation of the vibration patterns of the tympanic membrane and the response of ossicular movement to the stimulus of sound — an undulating traveling wave. In the decades since his claim to the Nobel Prize in 1962, there has been an explosion of knowledge about morphology and function that has produced what seems to be an endless series of surprising and intriguing results. Incidentally, von Békésy's discoveries stand fast; only minute details of his findings are being investigated As this book shows, the sensory transduction in the cochlea has attracted more research effort than any other part of the auditory system. Such is the reason that this book is heavily weighted toward chapters on cochlear physiology. The anatomy and physiology of the ear and auditory nervous system are clearly presented and, for the most part, can be understood with minimal knowledge of the physics of sound. Chapter 19 has some of the best and some of the worst; that is, it provides an excellent understanding of how the auditory system works, but for the clinician, it provides too much information on physics.

There are several issues of interest, one being that of cell coupling, which has been used to explain potassium transport through the cell and abnormalities that cause genetic hearing loss. There is an impression that there is no direct indication of this function (page 386). Of course, one has to accept the fact that there will be certain experimental uncertainties and, at times, diverse opinions concerning certain critical issues.

A book of such magnitude dedicated to physiology has to have a good index system if it is to be used as a reference book. The index *is* good; if I wanted to read about the expansion characteristics of the outer hair cells (a new discovery by W. E. Brownell et al in 1985), for example, the topic is available in 3 different sections. There are only scant indexed listings on hereditary hearing disorders, probably because the editors felt that these were more apropos to the field of molecular medicine.

Applicability: Every medical specialty needs standard reference textbooks — resources to which students, residents, fellows, specialists, researchers, and other interested practitioners may turn for reliable information. Such a text in this field is the second edition of *Physiology of the Ear*.

DENNIS G. PAPPAS, MD
Birmingham, Alabama

Genetics and Hearing Loss

Charles I. Berlin and Bronya J. B. Keats, editors. Hard cover, illustrated, indexed, 133 pages, 2000. Singular, San Diego, Calif, $71.95.

Purpose: This book presents the results of a genetics studies symposium held September 27, 1998. Presenters gave results of their research in the genetics of hearing and also reviewed studies ranging from identification and characterization of genes involved in hearing impairment to the potential for effective therapies based on knowledge of gene structure and function.

This is the fifth in a series of books in the Kresge-Mirmelstein Award cycle. All of these books have been published and supported by Singular Publishing Group, and typically, they have been edited by Dr Berlin.

Contents: There are only 7 chapters, and at least half of the book reports the use of mouse models and involvement of hereditary hearing impairment in humans. Chapters include various techniques and modalities such as "Physical Maps as Molecular Tools to Identify Disease Genes," "Gene Therapy for the Treatment of Hearing Disorders," and "Transmitters in the Cochlea: ATP as a Neuromodulator in the Organ of Corti." The remaining short chapter portrays an "Adaptation to Deafness in a Balinese Community"; a CD-ROM accompanies this chapter.

Attributes and Limitations: The word "mutations" is a common one in the field of genetics, and such is the case in this book. It brings to mind the work of Hermann Joseph Muller, who contributed much to the foundations of genetics. It was he who proposed that the gene was the basis of life, and that each gene serves as a template for its own replication. He described the occasional changes in genes — mutations — and found that these alterations persisted throughout successive generations. For his far-reaching discovery that mutations were produced by exposure to x-rays, he received the Nobel Prize in 1946.

The articles provided in this small volume give useful information, especially for the clinician, in that they afford insights into the mechanisms of action of the known disorders of mutant genes that cause prenatal and, probably, postnatal deafness. As of late, relevant information concerning genetics has been plentiful. This is not a standard reference textbook on genetics for the otolaryngologist; rather, the text dwells on the physiology and location sites of such genes as GJB2, Myo7a, myosin IV, PDS (Pendrin), and ATP6B1. Excellent insights are given into the distinctive roles of these genes and their effects upon the development of the membranes of the cochlea. Many of these anatomic and physiological findings have been made with the scanning electron microscope. This work is clearly presented and may be understood with minimal knowledge of research details.

Some of the remaining chapters are difficult to read. I suppose that the scientific progress reports make for such heavy going.

Applicability: Clinicians, clinical researchers, and basic scientists in genetics may turn to this book to better apply genetics science for improving their knowledge on hearing loss.

DENNIS G. PAPPAS, MD
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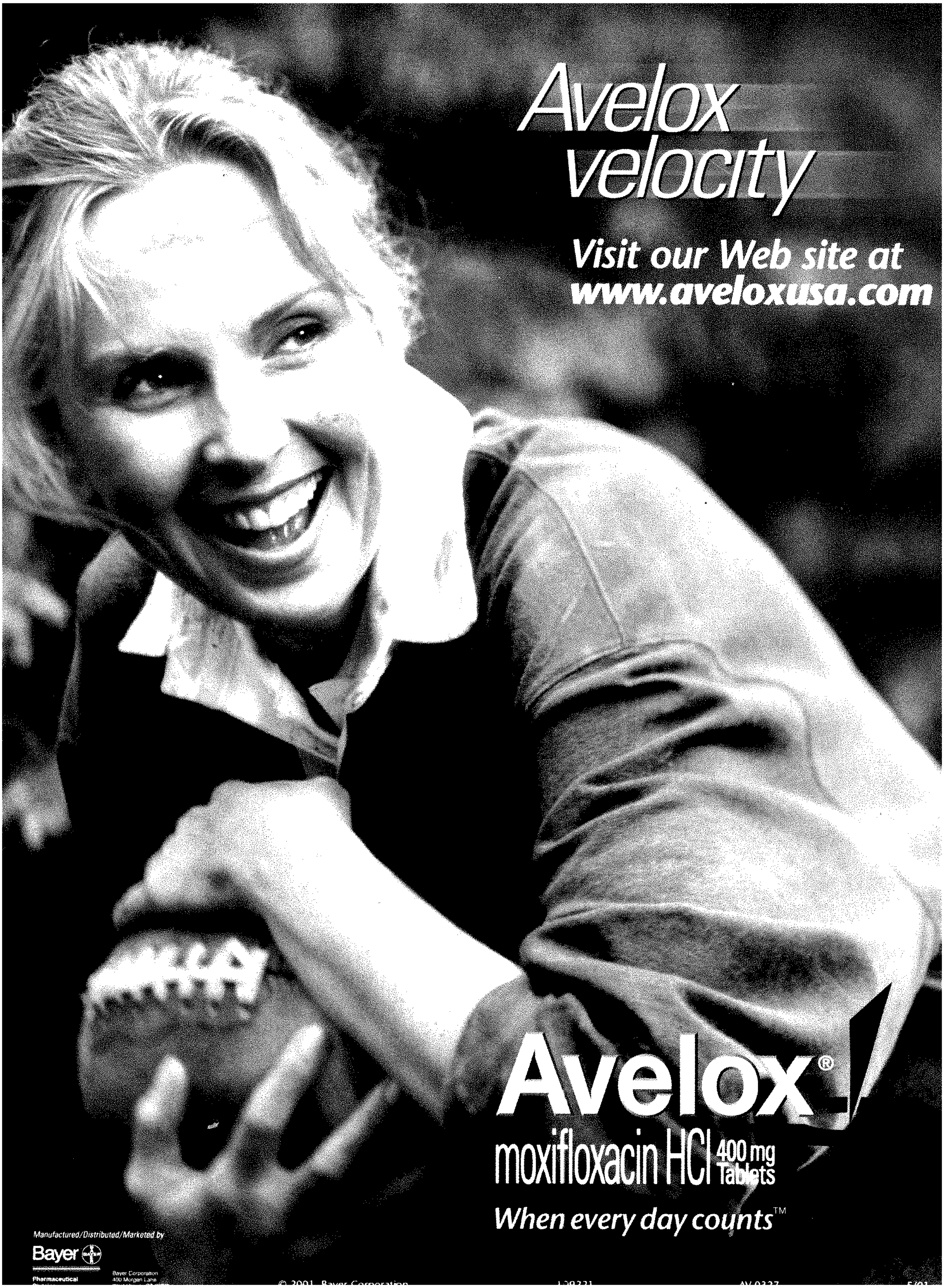
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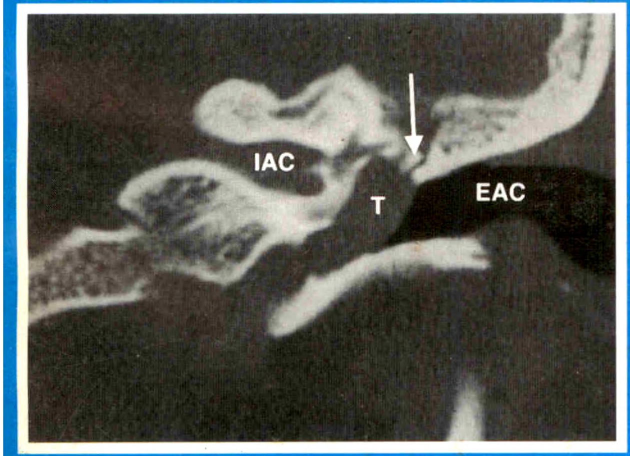

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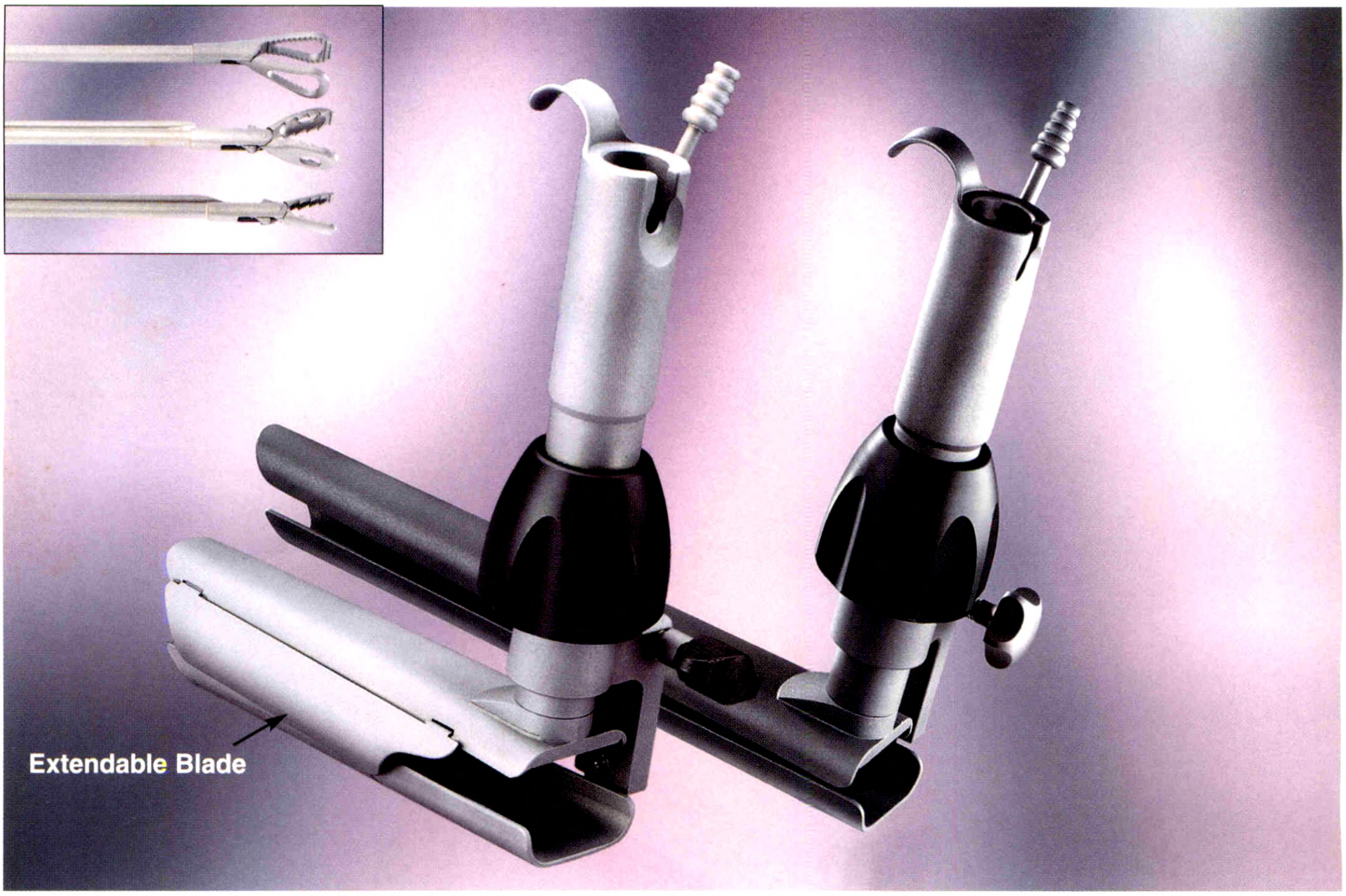

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EVALUATION OF POLY-L-LACTIC ACID AND POLYGLYCOLIC ACID RESORBABLE STENTS FOR REPAIR OF TRACHEOMALACIA IN A PORCINE MODEL

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Poly-L-lactic acid and polyglycolic acid (PLPG) resorbable stents may offer a potential solution to the problem of tracheomalacia. Advantages of this material include its strength, its versatile shaping characteristics, and its resorbability, which would preclude surgical removal and allow for airway growth. The purpose of this pilot study was to examine the usefulness of PLPG stents for temporary external airway stenting of tracheomalacia in a porcine model. A severe tracheomalacia was created in 6 pigs by submucosal resection of segments of tracheal cartilage from 6 consecutive rings. The PLPG stent was then shaped to recreate the tracheal contour and sutured to the underlying airway. Endoscopic photodocumentation during spontaneous ventilation was obtained before and after reconstruction. After creation of the malacic tracheal segment, all animals developed stridor, retractions, and cyanosis during spontaneous ventilation. After repair, all animals were extubated without complication. All animals survived the follow-up period of 9 to 12 weeks without evidence of respiratory distress and with rapid weight gain. Repeat bronchoscopy showed no evidence of airway collapse during spontaneous ventilation. Tracheal measurements revealed growth of the stented segment with a mild narrowing within the repaired region. Histologic examination showed preservation of respiratory epithelium. These preliminary findings suggest that PLPG stents may serve a useful role in the surgical management of tracheomalacia.

KEY WORDS — airway stent, resorbable stent, tracheobronchomalacia, tracheomalacia.

INTRODUCTION

Tracheomalacia is a congenital or acquired condition characterized by tracheal cartilage flaccidity that results in airway collapse during expiration. Symptoms range from mild expiratory stridor to apneic episodes with cyanosis and bradycardia. In mild pediatric cases, this problem may resolve without intervention as the trachea grows larger, firmer, and less prone to collapse over time. However, in more severe cases, surgical intervention is required, as the associated airway collapse can lead to progressive respiratory compromise and a potentially fatal outcome.[1-3]

Traditional methods of tracheomalacia repair include aortopexy and tracheal resection. Although effective under certain conditions, these techniques possess significant limitations. The use of aortopexy is limited to situations in which the malacic segment is opposite the aorta. Tracheal resection is only applicable to very short malacic segments and is often complicated by stenosis at the anastomosis site.[1-4]

Within the past decade, external and internal airway stenting techniques have been attempted for tracheomalacia repair, but they have not proven to be consistently reliable. Limitations of current methods of stenting include the lack of a shape-memory material that is easily inserted, accommodates airway growth in children, is nonreactive to tissues, and is easily removed. Because of the small size of the pediatric airway, internal stenting has been associated with severe complications, including stent migration and death.[4-7] The lack of a suitable graft material, synthetic or autologous, has limited the efficacy of external stenting.[5,6]

An absorbable material would allow for stenting of the trachea early in life without the creation of a long-term impediment to tracheal growth. Further, an absorbable material would preclude the need for

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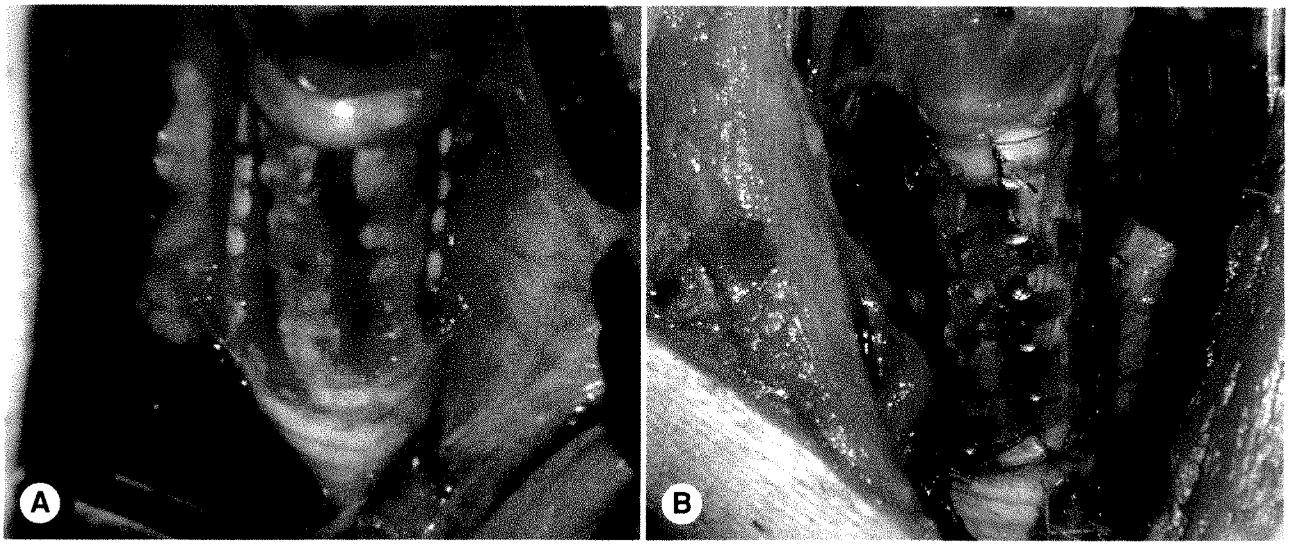

Fig 1. Operative procedure. **A)** Creation of malacic segment by submucosal resection of 3 interrupted segments of tracheal cartilage from 6 consecutive rings (rings 2 through 7). Care was taken to avoid violation of tracheal mucosa. **B)** Repair of malacic segment by securing resorbable stent (poly-L-lactic acid and polyglycolic acid) to underlying airway with polyglycolic acid suture. Rings represent prefabricated holes within stent through which sutures were placed to secure stent to anterior tracheal wall.

surgical removal and would avoid the presence of a long-standing foreign body capable of infection or erosion. In the pediatric population, a temporary stent appears ideal, as the patient is likely to develop increased tracheal stability with age. Poly-L-lactic acid and polyglycolic acid (PLPG) resorbable plates may offer a solution to the problem of tracheomalacia. The promising qualities of this material include its strength, versatile shaping characteristics, and resorbability, which would allow for temporary stenting of the trachea while accommodating airway growth. The purpose of this study was to determine the efficacy of PLPG plates for temporary external airway stenting of tracheomalacia in a porcine model. This study represents a novel use of the PLPG material.

MATERIALS AND METHODS

Six pigs weighing 16 to 25 kg were used. The pig was selected as the animal model because of its established use in previous studies of tracheomalacia and its rapid airway growth.[4,8] Rapid airway growth in this animal model allowed for examination of the efficacy of surgical repair after substantial increases in tracheal diameter had occurred. All animals were treated in accordance with guidelines established by the University of Wisconsin Research Animal Resources Center.

Creation of Model for Tracheomalacia. Sedation was achieved by intramuscular injection of telazol (50 mg/mL tiletamine and 50 mg/mL zolazepam), xylazine hydrochloride, and atropine sulfate. The pig was then endotracheally intubated, and general anesthesia was achieved with inhalational isoflurane. The trachea was exposed via a midline neck incision. The anterior and lateral aspects of the trachea were separated from the strap muscles, thyroid gland, and overlying soft tissues by blunt dissection. Measurements of the external tracheal diameter were obtained at rings 1, 4, and 8.

Model creation was based on a previous study by Mair et al,[4] in which resection of 5 consecutive tracheal rings resulted in a fatal malacia. In our study, a malacic tracheal segment was created by the submucosal resection of 3 interrupted segments of cartilage (Fig 1A) from 6 consecutive tracheal rings (rings 2 through 7). Segments of cartilage were maintained within the malacic region, in order to create a model that more closely resembled pediatric tracheomalacia, in which tracheal cartilage is present but lacks adequate strength to prevent airway collapse.

The pig was then examined during spontaneous ventilation, and endoscopic photo and video documentation of airway collapse was obtained. Photo documentation was achieved with the use of a flexible endoscope through the endotracheal tube after withdrawal of the tube to a point proximal to the malacic segment. The severity of tracheomalacia was documented by the presence of stridor, retractions, and cyanosis during spontaneous ventilation. Mechanical ventilation and general anesthesia were then resumed.

Repair With PLPG Resorbable Stent. A PLPG (Walter Lorenz Surgical, Inc, Jacksonville, Florida) mesh panel (50 × 50 mm, 0.75-mm thickness) was cut to a size slightly larger than the malacic tracheal

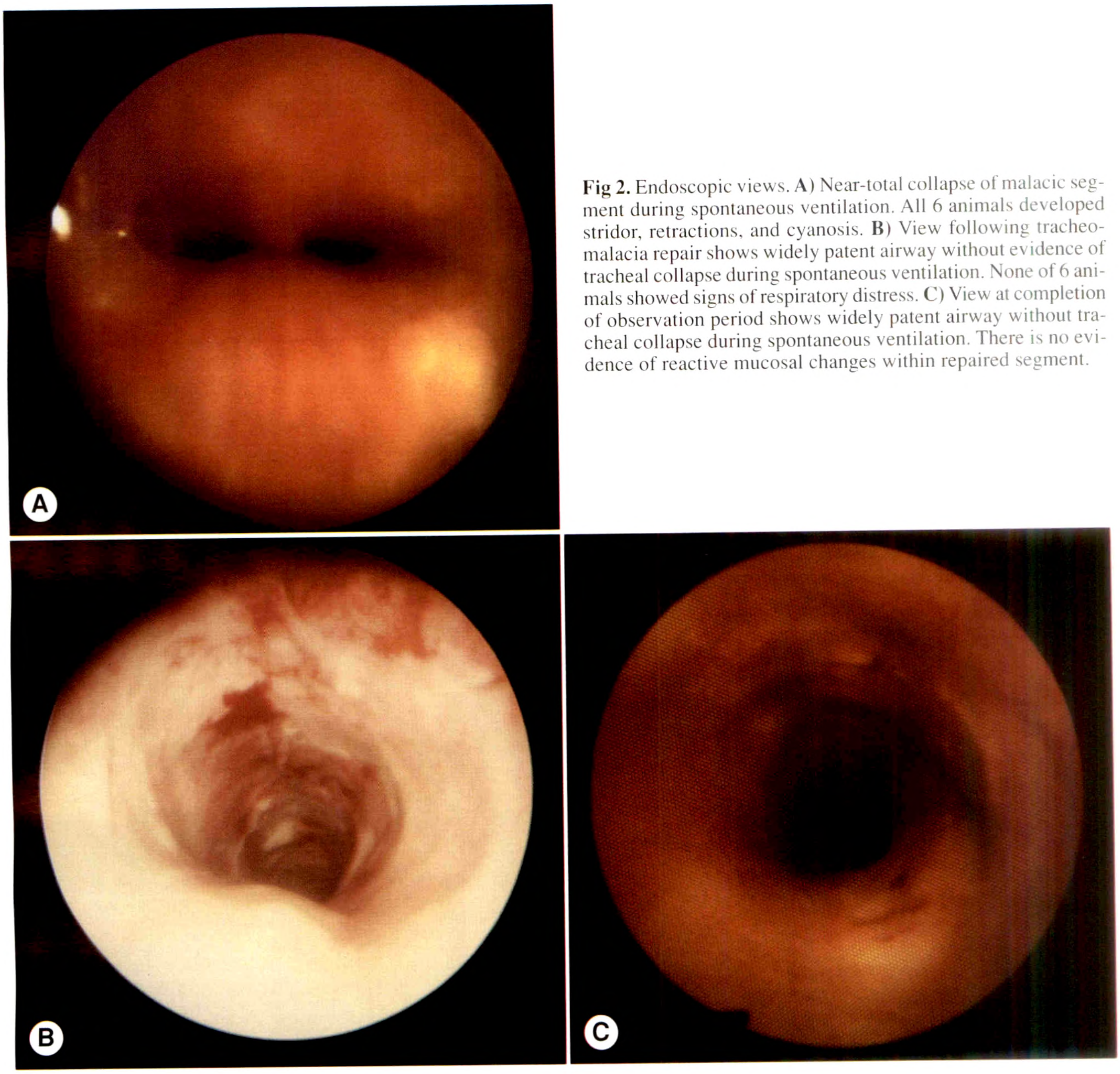

Fig 2. Endoscopic views. **A)** Near-total collapse of malacic segment during spontaneous ventilation. All 6 animals developed stridor, retractions, and cyanosis. **B)** View following tracheomalacia repair shows widely patent airway without evidence of tracheal collapse during spontaneous ventilation. None of 6 animals showed signs of respiratory distress. **C)** View at completion of observation period shows widely patent airway without tracheal collapse during spontaneous ventilation. There is no evidence of reactive mucosal changes within repaired segment.

segment. The plate was fashioned into a U shape in order to recreate the normal tracheal contour. By means of 4-0 and 5-0 absorbable polyglycolic acid suture material, the stent was then secured to the underlying airway (Fig 1B). The airway was reexamined during spontaneous ventilation, and endoscopic photo and video documentation was obtained as previously described. The animal was reevaluated for the presence of stridor, retractions, or cyanosis during spontaneous ventilation.

The animals were followed for 9 to 12 weeks after the procedure. Daily evaluation for signs of respiratory distress and oral intake was performed, and weight gain was recorded. At the completion of the observation period, repeat bronchoscopy with photo and video documentation of the repair site was performed during spontaneous ventilation. The animal was then euthanized, and the larynx and cervical trachea were harvested. Measurements of the outer and inner tracheal diameter at rings 1, 4, and 8 were obtained.

The tracheas were then fixed in 10% neutral buffered formaldehyde and sectioned in the sagittal and transverse planes. Decalcification in acidic solutions did not soften the prosthetic material, the trabeculae of which were removed from fixed tissue sections with a fine forceps. Tissue sections, 5 μm in thickness, were then stained with hematoxylin and eosin and examined by light microscopy.

RESULTS

Respiratory Signs. After creation of the malacic

TABLE 1. DURATION OF POSTOPERATIVE OBSERVATION PERIOD AND ANIMAL WEIGHT GAIN

Pig	*Observation Period*	*Weight Gain (kg)*
1	9 wk 3 d	25.4
2	10 wk 5 d	26.8
3	10 wk	26.1
4	12 wk	43.2
5	10 wk	26.8
6	9 wk	25.5

segment, all 6 pigs developed stridor, substernal and intercostal retractions, and cyanosis during spontaneous ventilation. Endoscopic examination revealed near-total collapse of the involved tracheal segment in all 6 animals (Fig 2A). After repair, repeat endoscopy revealed the absence of tracheal collapse during spontaneous ventilation (Fig 2B). All animals were successfully extubated without evidence of postoperative respiratory distress. The follow-up period ranged from 9 to 12 weeks. During this period, there was no evidence of respiratory distress upon daily examination of all 6 animals, and all animals showed rapid weight gain (Table 1). Follow-up endoscopic examination revealed the absence of tracheal collapse during spontaneous ventilation and no evidence of reactive changes within the tracheal lumen (Fig 2C).

Tracheal Growth. At postmortem examination, tracheal rigidity was found at the repair site (Fig 3A). Outer-diameter measurements of tracheal rings 1, 4, and 8 (corresponding to levels above, at, and below the repair site) revealed substantial growth of the repaired segment with a slight narrowing as compared to surrounding normal trachea (Table 2). Inner-diameter measurements at these levels revealed a narrowing within the repaired region ranging from 2 to 4 mm (Figs 3B and 4).

TABLE 2. OUTER TRACHEAL DIAMETERS BEFORE OPERATION AND AT END OF OBSERVATION PERIOD

Pig	*Tracheal Ring*	*Preoperative Diameter (mm)*	*Final Diameter (mm)*
1	1	14	21
	4	14	20
	8	14	22
2	1	14	22
	4	15	20
	8	14	22
3	1	14	20
	4	14	18
	8	14	19
4	1	15	21
	4	16	20
	8	16	22
5	1	14	21
	4	14	20
	8	14	21
6	1	14	20
	4	15	19
	8	15	20

Histopathologic Analysis. Upon histopathologic examination, the tracheal wall in the area of the implant was more rigid than normal fixed trachea. Tracheal ulceration, fissures, or protrusion of prosthetic material into the lumen was not seen. Microscopic examination showed empty spaces, previously occupied by the trabeculae of the PLPG stent, external to the tracheal cartilage segments. These angulated spaces were lined by myofibroblastic and collagenized fibrous tissue with a scant lymphocytic infiltrate (Fig 5A). The thickness of this fibrous layer varied from 0.25 to 2.0 mm. The submucosa contained the normal areas of adipose tissue, smooth muscle components, and seromucinous glands. In many sections, a normal epithelium with abundant cilia was

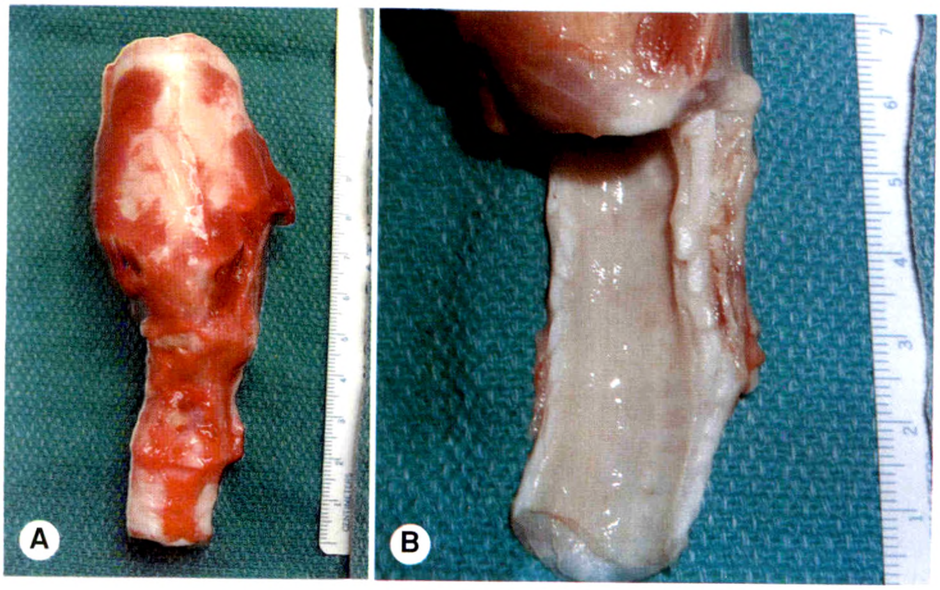

Fig 3. Harvested tracheas. **A)** Rigidity of repaired segment with mild narrowing of outer tracheal diameter. **B)** Trachea divided in coronal plane shows mild narrowing of inner tracheal diameter.

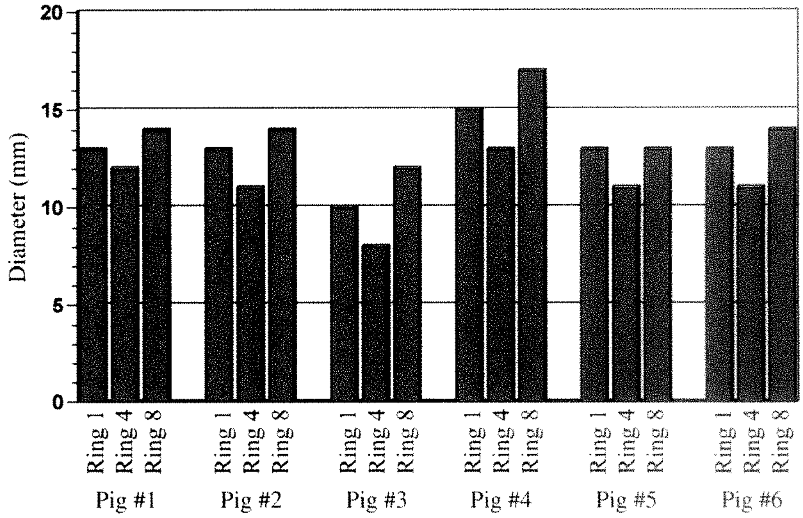

Fig 4. Inner tracheal diameters at completion of observation period.

present (Fig 5B). In some areas, the mucosal epithelium was flat and attenuated to 3 cell layers in thickness; however, some cilia were still evident in most of these sampled areas. Squamous metaplasia was not seen.

Complications. During creation of the tracheomalacia model, there were 2 animal deaths due to severe postoperative wound infections. These deaths occurred on postoperative days 6 and 7. In both cases, the animals were witnessed having contaminated their incision lines after removing their surgical dressings or having their dressings removed by other animals. There was no evidence of respiratory distress in either animal before death, and examination of the airway during necropsy revealed a secure stent without evidence of airway collapse. As a result of these deaths, additional measures were taken to protect the surgical wound, including the use of multiple layers of dressings, postoperative broad-spectrum antibiotics, and housing of the animal in an isolated environment for 1 week after surgery. After institution of these measures, no further wound infections or deaths were encountered.

DISCUSSION

In the majority of cases, tracheomalacia in the pediatric population is mild, and it is often managed conservatively. In severe cases, however, surgical intervention is required.[1,2] Because of the limitations of aortopexy and tracheal resection, airway stenting has gained increased attention for the management of severe tracheomalacia. However, both external and internal stenting have been associated with significant limitations that have precluded routine clinical application for this condition.

Several forms of internal stenting for tracheomalacia have recently been evaluated in animal models, including woven polymeric stents, stainless steel "zigzag" stents, and thermal shape-memory titanium-nickel alloy internal stents.[4,6] However, some of these methods were associated with less-than-optimal outcomes, such as severe respiratory obstruction with inflammation and tracheal stenosis, and animal death due to stent migration.[4] Further, histopathologic examination showed squamous metaplasia of the stented lumen.[4,6] Metallic angioplasty stents have also been implanted in the trachea and bronchi of pediatric patients, with good results in 4 of 7 children over a mean follow-up duration of 11 months.[9] In all of these studies, the effects of internal stenting on the growth of the trachea were not quantified.

External stenting has included the use of polytetrafluoroethylene grafts[7] and Marlex mesh secured by suture or fibrin glue.[10] These stents have also been associated with complications, including death due to graft erosion and fixation of the trachea around its entire circumference.[7,10] As a result, these techniques have not gained widespread acceptance for the surgical management of pediatric tracheomalacia.

Despite the problems associated with prior attempts at stent application for tracheomalacia, it is encouraging that there have been reports of good outcomes after stenting in some patients.[6,9,10] These reports suggest that the use of a dependable stent may provide a solution to the problem of tracheomalacia. The ideal pediatric airway stent would possess the following features: 1) adequate strength to prevent airway collapse, 2) technical ease of placement, 3) accommodation of future airway growth, 4) lack of significant tissue reactivity, and 5) absorbability, precluding the need for a second procedure for removal. In our model, PLPG resorbable plates exhibited all of these features. Further study is required to determine the applicability of this model to the human airway.

In this pilot study, the use of PLPG resorbable plates as external stents resulted in the immediate repair of a potentially lethal tracheomalacia in all 6

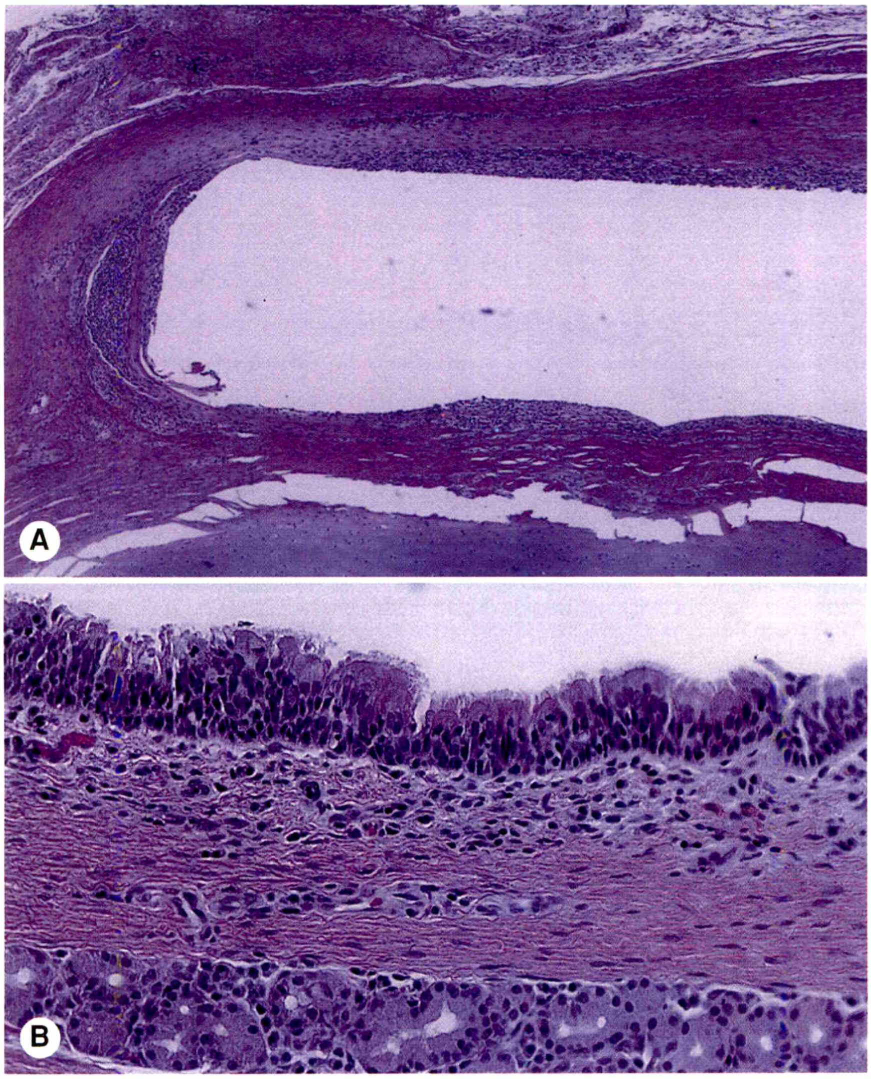

Fig 5. Histologic analysis of stented segment. **A)** Myofibroblastic and collagenized fibrous tissues surround spaces occupied by removed stent fragments (H & E, original ×20). **B)** Maintenance of ciliated respiratory epithelium within stented segment (H & E, original ×200).

animals. Stent placement was technically simple, and the stent was found to be stable, without evidence of migration or tracheal erosion. Tissue reaction within the trachea was minimal, and ciliated respiratory epithelium was largely maintained. These plates, typically used for craniomaxillofacial fixation, undergo progressive resorption with 90% loss of strength within 8 weeks, and complete resorption within 1 year (Walter Lorenz Surgical, Inc). We selected a follow-up duration of 9 to 12 weeks to evaluate the animals for the presence of recurrent malacia after loss of greater than 90% of stent strength. None of the 6 animals in this study showed signs or symptoms of recurrent malacia at the completion of the study period. Long-term studies are required to determine whether repair is maintained within the human airway until tracheal growth produces increased airway stability.

This study represents the first attempt to quantify the effects of tracheal stenting on airway growth. In our model, the inner tracheal diameter within the stented segment (ring 4) was 2 to 4 mm less than the diameter within normal rings above and below this segment (rings 1 and 8). This decrease in inner tracheal diameter ranged from 14% to 33% and was clinically insignificant, as all animals displayed an absence of respiratory distress and showed rapid weight gain — findings suggestive of good health and adequate respiratory support. This narrowing may be partially attributed to the rapid growth rate in our animal model. During the 9- to 12-week study period, these animals displayed an average weight gain of 29 kg and an average increase in outer tracheal diameter of 6.5 mm at ring 8. In a pediatric patient, the PLPG stent would be completely resorbed before such growth was achieved. Accordingly, less restriction of airway growth would be expected.

Congenital tracheomalacia is often characterized by widening of the membranous posterior wall in addition to cartilaginous instability. The porcine model used in this study could not recreate membranous wall widening. However, the model clearly results in tracheal collapse (Fig 2A) with approximation of the anterior and posterior tracheal walls as is seen in congenital malacia. In addition, tracheomalacia may involve long segments of the trachea and bronchi. Further studies are currently in progress to evaluate the usefulness of PLPG stents for long-segment tracheomalacia.

Tracheomalacia represents a spectrum of conditions with various degrees of severity; as a result, no single treatment method will be applicable in all cases. We believe that this technique represents an advancement in the management of tracheomalacia, and may also be applicable to other conditions requiring temporary airway stenting.

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ELECTROGLOTTOGRAPHIC AND ACOUSTIC CHANGES FOLLOWING TYPE I THYROPLASTY OR AUTOLOGOUS FAT INJECTION

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This study identified the electroglottographic (EGG) and acoustic measures that were most predictive of change in the glottic gap size of patients undergoing type I thyroplasty or autologous fat injection. Simultaneous EGG and acoustic recordings were made before and after autologous fat injection in 23 patients and type I thyroplasty in 45 patients. Relative measures of glottic gap size were obtained before and after operation from videostroboscopic images. Regression and correlation analysis was used to determine the strength of associations between the EGG or acoustic measures and glottic gap changes. A significant regression equation for both patient groups was constructed. The strongest predictors of glottic gap size change were changes in the EGG open quotient, signal-to-noise ratio, and mean fundamental frequency. Glottography provided a clinically useful means of evaluating glottic gap changes in patients undergoing vocal fold medialization procedures. Preoperative-postoperative differences in signal-to-noise ratio and mean fundamental frequency also were correlated to glottic gap size changes, but to a lesser degree.

KEY WORDS — autologous fat injection, electroglottography, glottic gap, type I thyroplasty.

INTRODUCTION

Glottic gap deficiencies are attributable to multiple causes. Among these are neuromuscular paralysis or paresis,[1] defects of the vocal folds secondary to microsurgical intrusion,[2] formal resection of a portion of the vocal fold secondary to tumor,[3] and natural aging processes such as presbylaryngis.[4]

Multiple procedures have been advocated to medialize the vocal folds to eliminate glottic defects. These include injection laryngoplasty with substances such as Gelfoam,[1] collagen,[5,6] autologous fat,[7-9] and Teflon.[10,11] Phonosurgical alternatives have been developed, including Isshiki type I thyroplasty and modifications employing various techniques with an external approach.[12-14] Microsurgical techniques such as re-neurotization and neurorrhaphy have been advocated.[15,16]

Regardless of the treatment method used to medialize the vocal fold, there is a continued focus on identifying the most useful method of assessing changes after treatment. Various techniques for documenting changes after medialization have been reported. Traditional methods have included perceptual ratings of vocal quality,[17] as well as more sophisticated acoustic and aerodynamic measurements.[18-20] The advent of videolaryngostroboscopy has facilitated evaluation of vocal fold mobility and glottic gap defects. With image analysis software, it is now possible to assess the extent of glottic gaps by means of relative measures. (There generally is not an object in the visual field that can be used as a reference for absolute measures.) However, measurements of the glottic gap are not frequently used clinically, because of the extensive time commitment needed for analysis and the frequently incomplete view of the glottis that is provided. The perceptual ratings have face validity, but the subjective nature of the task has called into question the reliability of such ratings.[17] The acoustic and aerodynamic measures have been useful to some extent, but there has not been sufficient study of the direct correspondence to the size of the glottic gap.[21]

Electroglottography (EGG), originally developed by Fabre in 1957, is an additional method of evaluating vocal fold function. It is appealing for use in patients with glottic gap deficiencies, because it provides information regarding the extent of vocal fold contact,[22,23] and thus it could be used before and after operation to document changes in this parameter. Electroglottography is relatively inexpensive, noninvasive, and technically feasible in clinical settings. However, additional documentation of the correspondence between EGG measures and glottic gap size is needed. We evaluated 2 series of patients with glottic gap deficiencies (those with unilateral vocal fold paralysis and those with volume deficiencies of the vocal folds) using various EGG and acoustic measures. We then calculated the degree of correlation between each measure (individually and relative to

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TABLE 1. SUBJECT GROUP DEMOGRAPHICS

	Autologous Fat Injection	*Type I Thyroplasty*
No. of patients	23	45
Sex	61% M 39% F	53% M 47% F
Mean age (y)	54 (range, 27-64)	61 (range, 48-82)
Vocal fold disorder	Vocal fold mass excision in 15, vocal fold scarring in 8	Idiopathic paralysis in 21, thyroidectomy in 13, skull base tumor excision in 8, stroke in 3

one another) and glottic gap size. The intent was to determine which of the group of measures might best serve as an indication of glottic gap change following surgical intervention.

MATERIALS AND METHODS

SUBJECTS

Two groups of patients were evaluated. The first consisted of 23 patients who underwent unilateral autologous fat injection into the vocal fold at the University of Kansas Medical Center, Voice and Swallowing Disorders Center, between August 1991 and August 1997. The second group comprised 45 patients who had undergone vocal fold medialization via type I thyroplasty during the same time period. Specific patient demographics and information are provided in Table 1. In general, those patients who ultimately underwent the injection procedure had fairly small gaps (<5 mm), while those who underwent the thyroplasty procedure exhibited relatively larger gaps (>5 mm) as estimated from videolaryngostroboscopy. All surgical procedures were performed by the senior author (G.Y.S.). Autologous fat injections were performed in the manner previously described.[9] Type I thyroplasty was performed with a custom-carved silicone block in the manner described by Wanamaker et al.[24]

DATA COLLECTION

Measurements were obtained on 3 occasions: before operation, 3 months after operation, and 6 to 9 months after operation. Ultimately, the analysis included only the overall change from the preoperative state to the final measurement at 6 to 9 months, as the 3-month postoperative measurement closely paralleled the final measurement. At each visit, several measurements were recorded as described below. Additionally, 3 patients not part of either group described above were submitted to EGG during type I thyroplasty procedures so we could gather preliminary data on the feasibility and usefulness of such measures.

Glottic Gap Measurement. Videolaryngostroboscopy was performed with a Kay 70° rigid telescope (Kay Instruments, Lincoln Park, New Jersey, model SN0296) attached to a CCD camera (Toshiba, Osaka, Japan, model IK-C30A) and a stroboscopic light source (Bruel and Kjaer, Marlboro, Massachusetts, model 4914). Recordings were made with a Super VHS recorder (Mitsubishi, Cypress, California, model BV-1000). The video signal was viewed on a high-resolution monitor (Sony, Tokyo, Japan, model PVM-1343MD). After the telescope was positioned, the patient was asked to produce a sustained /i/ vocalization at a comfortable, self-determined pitch and loudness. Stroboscopy was performed and recorded.

The video image was displayed on the monitor and moved frame by frame until maximum glottic closure occurred. Care was taken to sample a number of portions of the recording to ensure that maximum closure was being observed. Only the gap bordered by the anterior two thirds of the vocal fold (ie, anterior to the tip of the vocal process) was used for measurement; this practice facilitated measurement in larynges with prolapsed arytenoid cartilages. Patients with prolapsed arytenoid cartilages obscuring the vocal process tip were not considered for this study. Additionally, care was taken that images used for analysis were made with the scope in midline, so that unusual image angles were avoided. The image of maximum closure was captured by image analysis software (Image Pro Plus, Sacramento, California) loaded on a personal computer. The software's auto-tracking subroutine was engaged to trace the edge of each vocal fold; a line was drawn across the glottis from one arytenoid tip to the other, and the number of computer screen pixels within the circumscribed area was obtained. The pixel measure is not an absolute measure of glottic area. Additionally, if the height of the scope relative to the vocal folds varied within or between stroboscopic studies, even direct comparison in pixels would be inappropriate. To help control this problem, relative glottic gap sizes were calculated by also obtaining a measure of the glottic gap present when the cords were maximally abducted. The ratio of the glottic gap measured during maximum adduction to that measured during abduction was then calculated. An additional precaution used within a stroboscopic study was to take the maximum abduction image that was closest to the maximum adduction image used, so that the scope positions were most likely comparable.

Electroglottography. Electroglottographic recordings were made simultaneously with the stroboscopy. The electrodes of a 2-channel electroglottograph (Glottal Enterprises, Syracuse, New York, model MC2-1) were place on either side of the thyroid alae

TABLE 2. REGRESSION EQUATIONS AND STATISTICAL RESULTS BY SUBJECT GROUP

	Glottic Gap Regression Equation	*% Variance Accounted For*	*t Value**	*p*
Autologous fat injection	$\Delta\text{Gap} = 0.543 + 0.841\ (\Delta\text{OQ}) + 0.384\ (\Delta\text{SNR}) + 0.265\ (\Delta\text{mean } F_0)$	92.49	2.46	.008
	[70.72%] [14.75%] [7.02%]			
Type I thyroplasty	$\Delta\text{Gap} = 0.311 + 0.796\ (\Delta\text{OQ}) + 0.436\ (\Delta\text{SNR}) + 0.231\ (\Delta\text{mean } F_0)$	87.71	2.08	.016
	[63.36%] [19.01%] [5.34%]			

Δ — Change from before medialization to after medialization; OQ — open quotient; SNR — signal-to-noise ratio; F_0 — fundamental frequency.

*For slope of regression line.

and were held in place with an elastic collar. The glottographic signal was routed to a signal processing board (SA Instrumentation, Encinitas, California, model SA-415), on which it was low-pass-filtered at 1,500 Hz and routed to a Data Translation I/O board (Marlboro, Massachusetts, model 2821), which fed the signal to a personal computer. C-Speech software (version 3.1, Milenkovic, Madison, Wisconsin) was used to display, mark, and measure the glottographic waveform. The signal was differentiated and displayed with the original waveform. The differentiated signal was used to help mark the point of initial glottic opening, the point of peak opening, and the point of closure as described by Murty et al[25] and Hanson et al.[26] From these points, 2 measures of interest were obtained: the open quotient (OQ; time vocal folds are open divided by total time of glottic cycle) and the speed quotient (SQ; opening time divided by closing time). The mean OQ and SQ were calculated from 5 representative cycles from the middle 100 ms of the vowel production from which the glottic gap image was taken. Electroglottography (and the quotients obtained from the signal) has been used by a number of investigators to assess patients with vocal fold paralysis.[27,28] Sercarz et al stated that "the differentiated electroglottographic waveform provides useful information about the timing of glottic opening and closure in states of asymmetric laryngeal vibration."[27(p567)]

Three patients who underwent unilateral type I thyroplasty had glottographic measures performed in the operating room just before and immediately after implant insertion to reduce the effects of swelling.

Acoustic Recordings. Acoustic recordings were taken simultaneous with EGG and stroboscopy. Recordings were made in a quiet clinical suite with a Telex headset microphone (Evanston, Illinois, model PH-91) and a Marantz recorder (Chadsworth, California, model PMD-201). A constant mouth-to-microphone distance of 1.5 inches (3.8 cm) was maintained (with the microphone at the corner of the mouth to allow placement of the stroboscope in the mouth).

Tape-recorded signals were routed from the Marantz recorder to the analog signal processing board and I/O board mentioned above, and ultimately to a personal computer. Again, C-Speech software was used to display and analyze the signal. The middle 500 ms of the vowel production was used for analysis. The following measures were obtained: fundamental frequency (F_0), jitter (frequency perturbation), shimmer (amplitude perturbation), and signal-to-noise ratio (SNR). Additionally, measures were taken from /i/ productions by patients producing their highest and lowest pitches possible, so that the F_0 range could be calculated.

ANALYSIS

The primary handling of the data was accomplished by multiple regression and correlation analysis. Separate regression equations were calculated for the two patient groups, as the intent was not to compare the surgical procedures, but to simply identify those measures within each group that are most highly correlated with glottic gap changes. For the multiple regression analysis (Statistical Package for the Social Sciences, version 8.0, Chicago, Illinois), the change in the glottic gap ratio from the preoperative measure to the final postoperative measure served as the dependent variable. The predictor, or independent, variables were the changes from the preoperative measure to the postoperative measure in OQ, SQ, mean F_0, F_0 range, jitter, shimmer, and SNR. Essentially, the regression procedure determines how much of the variance in the glottic gap ratio change is accounted for (or predicted) by the independent variables. The technique allows for an assessment of the strength of the correlation between changes within each individual measure and the glottic gap ratio change as well. In this way, it can be determined which of the variables under study is most strongly correlated with glottic gap change. The t-test for the slope of the regression line was used to determine the statistical significance of the equation calculated.

RESULTS

For both subject groups, statistically significant regression equations were found (Table 2). The equa-

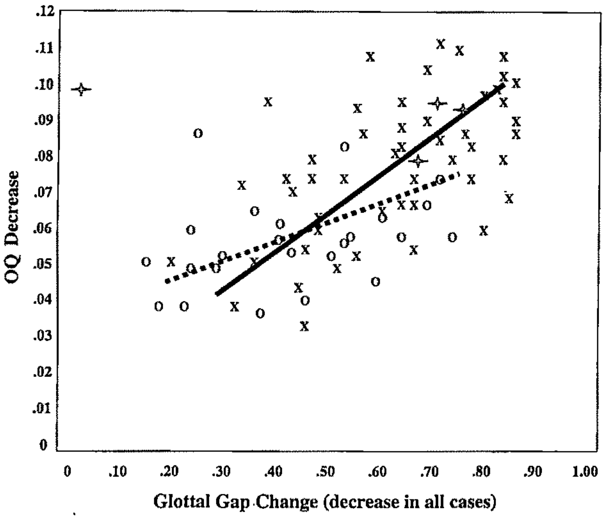

Fig 1. Plot of change in open quotient (OQ) versus ratio showing change in glottic gap for each subject (O — autologous fat injection subjects; X — type I thyroplasty subjects; star — intraoperative measurements in patients undergoing type I thyroplasty). Solid line is plot of line of best fit for type I thyroplasty group; dashed line is same for autologous fat injection group.

tions for the 2 groups paralleled one another in terms of the variables retained and the order in which they contributed to prediction of the change in the glottic gap ratio. This finding would suggest that the equations are fairly robust in predicting a change in glottic gap size, regardless of the surgical technique used. The overall regression equation for the type I thyroplasty group accounted for slightly more of the variance in glottic gap change than did the equation for the autologous fat injection group. The variables other than OQ, SNR, and mean F0 did not contribute significantly to the prediction of a change in glottic gap size in the stepwise regression procedure used here.

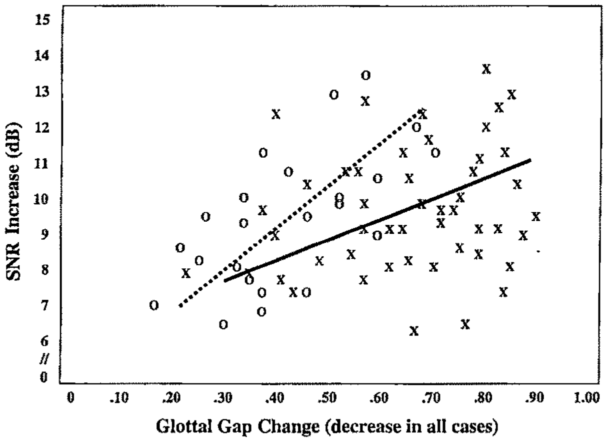

Fig 2. Plot of change in signal-to-noise ratio (SNR) versus ratio showing change in glottic gap for each subject (O — autologous fat injection subjects; X — type I thyroplasty subjects). Solid line is plot of line of best fit for type I thyroplasty group; dashed line is same for autologous fat injection group.

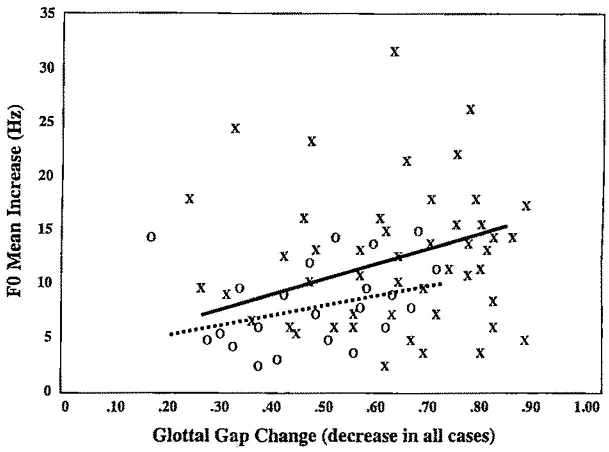

Fig 3. Plot of change in mean fundamental frequency (Fo) versus ratio showing change in glottic gap for each subject (O — autologous fat injection subjects; X — type I thyroplasty subjects). Solid line is plot of line of best fit for type I thyroplasty group; dashed line is same for autologous fat injection group.

Plots of the correlation between changes within each of the 3 significant variables and change in glottic gap size are provided in Figs 1-3. The full correlation matrix is offered in Tables 3 and 4 so that the strength of the intercorrelations among variables can be examined. There were some strong correlations among the independent variables. However, the fact that the intercorrelations among the 3 variables retained in the significant regression equation are fairly low suggests that each contributed something unique to the prediction of glottic gap size.

Because there are intraoperative data for only 3 subjects, statistical analysis was not completed. However, in each subject, the OQ measured during operation followed the trend observed in the subject pools. Plotting the OQ change by the glottic gap ratio change for these 3 patients yielded data points that fell near the regression line calculated for each variable (Fig 1).

DISCUSSION

As Berke et al[29] and Lin et al[30] have indicated, the clinical value of objective measures of vocal performance is not to diagnose, but rather to quantify changes as a function of treatment. If an objective measure can be identified that is able to document such changes, the measure could then be used to compare outcomes within and among treatment options for a given problem. To be most helpful clinically, the measure should be related to specific biomechanical aspects of vocal function, such as glottic closure. The intent of this study was to evaluate the ability of various EGG and acoustic measures to predict glottic gap size.

The results indicated that OQ change was the stron-

TABLE 3. CORRELATION MATRIX FOR ALL MEASURES FROM AUTOLOGOUS FAT INJECTION GROUP

	ΔGap	*ΔOQ*	*ΔSQ*	*ΔF0mean*	*ΔF0range*	*ΔJitter*	*ΔShimmer*	*ΔSNR*
ΔGap	1	**0.841**	0.136	**0.649**	0.277	0.218	0.301	**0.721**
ΔOQ		1	0.226	0.131	0.293	**0.468**	**0.509**	0.286
ΔSQ			1	0.376	0.013	0.098	0.214	0.331
ΔF0mean				1	**0.508**	0.332	0.190	0.167
ΔF0range					1	0.361	0.219	0.286
ΔJitter						1	**0.436**	**0.388**
ΔShimmer							1	0.363
ΔSNR								1

Bold denotes correlations significant at .05 level.
SQ — speed quotient.

gest predictor of change in glottic gap size for both subject groups. The strength of the prediction can be further increased by adding in measures of SNR and mean F0 change. The finding that glottographic measures are sensitive to change in glottic gap size is consistent with earlier reports of the validity of EGG measures.[31,32] The current study, however, also included comparison of the relative predictive power of other acoustic measures with the intent to narrow the clinical choices to the most effective measure(s).

Glottographic measures, particularly OQ, appear to be an ideal means of assessing changes in glottic closure after surgical intervention. The technique, while not a replacement for visually examining the vocal folds via stroboscopy, has several characteristics that make it appealing for clinical use: it is easy to perform, is inexpensive, is easily tolerated by patients, and provides a way to numerically analyze differences.

Electroglottography does suffer from certain drawbacks that limit its clinical usefulness. In some thick-necked individuals with significant pannus, the EGG signal is inconsistent. Some patients with a paralyzed vocal fold may begin to use the false vocal folds as a sound source. In this case, the EGG signal is unusable for the above purpose because any recorded closing phase most likely represents false fold phonation. This problem can also occur when a vocal fold has been successfully medialized but the patient persists in maladaptive false fold speech. While a trained ear can often detect this type of speech, it is generally necessary to correlate the EGG findings with laryngoscopic evaluation. Another issue for clinicians to be aware of is that changes in the EGG OQ that suggest improved glottic closure would be misleading in instances in which a paralyzed or scarred vocal fold is overcorrected. This overcorrection would result in a decreased OQ, but not necessarily an improvement in the voice. This underscores the need for acoustic analysis and a trained ear as part of the clinical armamentarium to recognize this occurrence and adjust the medialization accordingly, preferably during the operation.[33]

Two other variables contributed significantly to the regression equation, although to a lesser degree than the OQ change. A decrease in SNR following injection or surgery was not unexpected, as these procedures are essentially helping to reestablish more normal laryngeal function (ie, improved glottic closure during phonation). Others have reported an increase in the SNR following vocal fold medialization procedures.[34-36] The posttreatment F0 increase that was found in the present study has also been reported by others studying these and similar medialization procedures.[36-38] However, a lack of F0 change[39] and a decrease in F0[40] following medialization procedures have also been found. The discrepant findings across studies in terms of F0 change may be related to the specific subjects included within the various stud-

TABLE 4. CORRELATION MATRIX FOR ALL MEASURES FROM TYPE I THYROPLASTY GROUP

	ΔGap	*ΔOQ*	*ΔSQ*	*ΔF0mean*	*ΔF0range*	*ΔJitter*	*ΔShimmer*	*ΔSNR*
ΔGap	1	**0.738**	0.241	**0.663**	0.108	0.292	0.173	**0.581**
ΔOQ		1	0.358	0.152	0.202	**0.401**	0.311	0.199
ΔSQ			1	0.364	0.082	0.055	0.169	0.254
ΔF0mean				1	0.349	0.301	0.158	0.271
ΔF0range					1	0.344	0.197	0.155
ΔJitter						1	**0.419**	**0.425**
ΔShimmer							1	0.322
ΔSNR								1

Bold denotes correlations significant at .05 level.

ies. Before the vocal fold is medialized, some speakers may compensate for the glottic gap by tensing their vocal folds, thereby elevating pitch in the preoperative condition.[41] After vocal fold medialization in such cases, the pitch may drop to the more typical (ie, "noncompensated") level. However, not all individuals with a vocal fold paralysis engage in this pitch-elevating compensation before operation, and this difference may help explain some of the differing results along this acoustic parameter.

CONCLUSIONS

Electroglottographic measures of change in OQ provide a strong prediction of glottic gap size changes in patients who are to undergo type I thyroplasty or autologous fat injection. Because this measurement technique offers certain advantages over other measurement techniques, such as image analysis, acoustic measures, and perceptual ratings, we advocate inclusion of glottographic measures to assess the change in glottic gap size. Additionally, preliminary data from intraoperative EGG recordings suggest that the procedure may ultimately provide a means of assessing optimal vocal fold position during the actual medialization procedure, thus complementing vocal fold imaging and perceptual analysis.

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COSMETIC FACIAL SURGERY

The Indian Society of Facial Plastic and Reconstructive Surgery Inaugural Conference and Workshop on Cosmetic Facial Surgery will be held December 7-9, 2001, in Indore, India. For information, please contact Dr Subramaniam Iyyer (subu@md3.vsnl.net.in) or Dr Brajendra Baser, 109 Trade House South Tukoganj Indore MP 452001, India (brajendrab11@hotmail.com, baser@vsnl.com).

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PREVENTION OF ANTERIOR GLOTTIC RESTENOSIS IN A CANINE MODEL WITH TOPICAL MITOMYCIN-C

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To develop a model for recurrent anterior glottic stenosis and to test the efficacy of topical mitomycin-C in preventing restenosis, we induced anterior glottic stenosis with a CO_2 laser in 5 dogs. In 3 dogs, recurrence was established after surgical lysis. Subsequently, the 3 dogs received a single topical 3-minute treatment with a 1% solution of mitomycin-C after a second surgical lysis. In a parallel experiment, the other 2 dogs received a single topical 3-minute treatment with a 1% solution of mitomycin-C after the initial surgical lysis. An anterior glottic web was induced in all 5 dogs with the CO_2 laser. The 3 dogs experienced restenosis at the anterior glottis after surgical lysis alone. Mitomycin-C prevented anterior glottic restenosis in 2 of the 3 dogs treated twice and in both of the dogs treated once ($p = .02$). We conclude that a recurrent stenosis of the anterior glottis may be induced reproducibly in the canine model with the CO_2 laser. Application of topical mitomycin-C after lysis of an anterior glottic stenosis produces a statistically significant reduction in the rate of restenosis as compared to surgical lysis alone.

KEY WORDS — anterior glottic stenosis, glottic web, glottis, laryngotracheal stenosis, mitomycin-C.

INTRODUCTION

The sequelae of glottic mucosal injury may include impaired phonation, ventilation, and airway protection. In the anterior glottis, these debilitating and potentially life-threatening complications are commonly iatrogenic. At the root of these problems is exuberant granulation tissue formation and subsequent scarring that hinder the delicate workings of the larynx.[1] Many techniques have been devised to address unwanted scar and lesion formation in the anterior glottis. These treatment regimens are commonly undertaken only after the stenosis has already developed and the symptoms necessitate intervention. Current methods include intralesional injections, endolaryngeal procedures, and open procedures such as laryngofissure.[2]

Mitomycin-C is a naturally occurring antibiotic derived from *Streptomyces caespitosus* that has traditionally been used for intravenous chemotherapy. When given systemically, this compound is believed to exert antitumor activity through its ability to inhibit the synthesis of DNA at lower concentrations and its ability to inhibit the synthesis of RNA and proteins at higher concentrations.

In the field of ophthalmology, mitomycin-C has gained wide acceptance as a topical agent for procedures in which scarring and lesion re-formation are problematic. Its effectiveness is likely due to antiproliferative effects on cultured human fibroblasts at lower concentrations and cytocidal activity against these cells at higher concentrations.[3] Some common uses of topical mitomycin-C include the maintenance of patency after glaucoma filtration surgery[4,5] and dacryocystorhinostomy,[6] prevention of pterygium re-formation after primary excision,[7] and prevention of scarring after strabismus surgery.[8]

In the recent otolaryngology literature, the use of topical mitomycin-C has been explored as a means of reducing scar formation in both the posterior glottis[9] and the trachea.[10,11] The purpose of this study was to develop a model for recurrent anterior glottic stenosis and to test the efficacy of topical mitomycin-C in preventing restenosis in this area. It was hypothesized that mitomycin-C, used prophylactically, would reduce granulation tissue formation in the anterior glottis and thus reduce the incidence of recurrent anterior glottic stenosis.

MATERIALS AND METHODS

All research was approved and conducted according to the Institutional Animal Care and Use Committee (IACUC) guidelines at our institution. Five adult mongrel dogs weighing 15 to 20 kg were used. The animals were anesthetized with a mixture of atropine sulfate (0.05 mg/kg), butorphanol tartrate (0.05 mg/kg), xylazine hydrochloride (0.275 mg/kg induction, 0.2 mg/kg maintenance), and tiletamine hydro-

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From the Department of Otolaryngology, Vanderbilt Bill Wilkerson Center for Otolaryngology and Communication Sciences, Vanderbilt University Medical Center, Nashville, Tennessee. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Vanderbilt University.

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SUMMARY OF INTERVENTIONS AND OUTCOMES

Dog 1	*Dog 2*	*Dog 3*	*Dog 4*	*Dog 5*
Webbed	Webbed	Webbed	Webbed	Webbed
Lysed	Lysed	Lysed	Lysed	Lysed
Mitomycin	Mitomycin	No mitomycin	No mitomycin	No mitomycin
No web	No web	Web re-formed	Web re-formed	Web re-formed
		Lysed	Lysed	Lysed
		Mitomycin	Mitomycin	Mitomycin
		No web	No web	Web re-formed

Chronological sequence for each dog is read from top down.

chloride and zolazepam hydrochloride (4 to 6 mg/kg induction, 2 to 4 mg/kg maintenance). Exposure to the glottis was provided by a suspended Ossoff modified Pilling laryngoscope, remodified by application of a 2-cm extension to the anterior commissure portion of the laryngoscope to facilitate manipulation of the elongated canine epiglottis.

All canine larynges were exposed to 3 passes of a focused-beam CO_2 laser (Sharplan Lasers, Inc, Allendale, New Jersey) set at 10 W continuous mode. The lesion was induced from the anterior commissure to the vocal process, and from the ventricle to a point 2 mm below the edge of the vocal folds. This laser injury was then repeated at 2 weeks.

After notation of an anterior glottic stenosis (usually 2 weeks after the second laser injury), 2 dogs underwent simple laser lysis with the focused laser set to 10 W continuous mode. A 1% solution of topical mitomycin-C on a cotton pledget was set in contact with the laser lesion immediately and left in place for 3 minutes. The solution was not washed from the larynges after placement. To create the 1% solution, we mixed 5 mg of mitomycin-C powder (Bedford Laboratories, Bedford, Ohio) with 0.5 mL of sterile water. Follow-up laryngoscopy was performed every 2 weeks for 6 weeks. The experiments were all performed without blinding of the investigator-observer.

Two weeks after the second laser injury, the remaining 3 dogs underwent simple laser lysis of their anterior glottic stenosis without application of topical mitomycin-C. A pledget containing only saline solution was placed on the area of surgical lysis for 3 minutes, and follow-up laryngoscopy was performed every 2 weeks for 6 weeks. Recurrent stenoses were re-lysed, with application of a 1% topical mitomycin-C solution for 3 minutes without washing.

The animals were housed with water and a soft-mechanical diet for 24 hours after operation, and then were given water and regular dog food ad libitum until their sacrifice dates 6 weeks after the final laryngoscopy. Each animal was painlessly sacrificed with a lethal dose of concentrated pentobarbital sodium administered by intracardiac injection after induction of anesthesia.

The percent stenosis was determined from gross photographs of the larynx. Using digital images, we measured the length of the web from the anterior commissure to the vocal process. This length was put in ratio to the full length of the vocal fold. The investigator was not blinded to the treatment method when making this measurement.

Statistical analysis was performed by use of a Student's *t*-test to compare the 3 larynges treated with saline solution (the control group) to the 5 larynges treated with mitomycin-C (the experimental group).

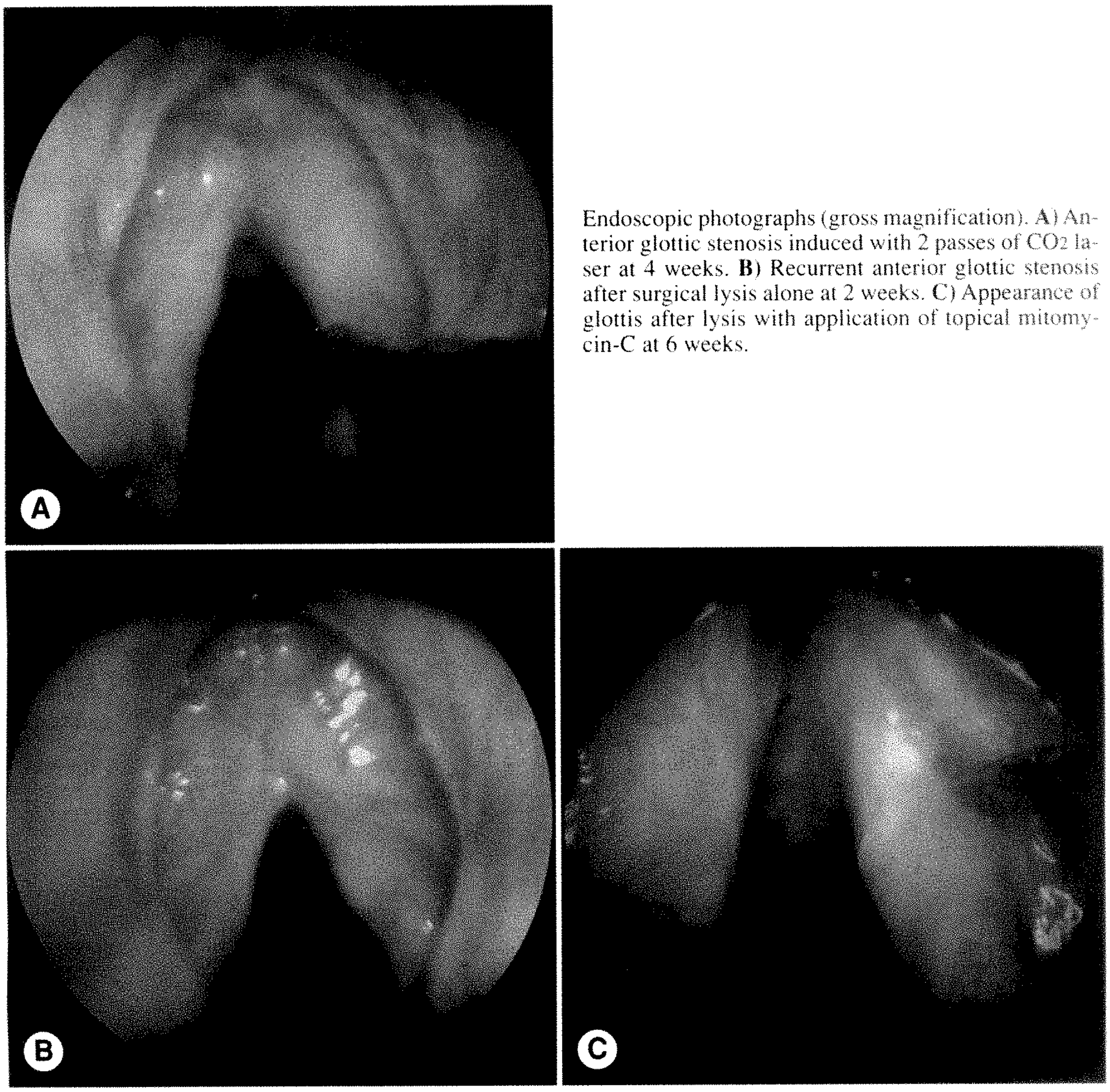
RESULTS

All animals survived to sacrifice. All wounds healed without infection, and no problems were encountered with local tissue reaction, decreased oral intake, airway compromise, or hemorrhage.

After the laser treatment to induce the anterior webbing, all 5 dogs demonstrated an anterior glottic stenosis ranging from 40% to 60% of the length from the anterior commissure to the vocal process. These lesions appeared 2 weeks after the second laser injury (4 weeks after the initial laser injury).

In the group of 3 dogs exposed to laser lysis of the anterior glottic stenosis alone, all 3 demonstrated restenosis at the anterior commissure of similar severity to the initial lesion. The restenosis occurred within 2 weeks in each case.

The experimental group was made up of the above 3 dogs with recurrent anterior glottic stenoses, as well as 2 dogs with previously unlysed stenoses. Four of these 5 dogs demonstrated no return of anterior glottic stenosis up to 6 weeks after surgical lysis and application of topical mitomycin-C (see Table). This was a statistically significant difference from the control group ($p = .02$). The 1 dog that had restenosis after topical mitomycin-C placement was from the group that had previously undergone surgical lysis alone. The lesion that recurred after treatment with



Endoscopic photographs (gross magnification). **A**) Anterior glottic stenosis induced with 2 passes of CO_2 laser at 4 weeks. **B**) Recurrent anterior glottic stenosis after surgical lysis alone at 2 weeks. **C**) Appearance of glottis after lysis with application of topical mitomycin-C at 6 weeks.

topical mitomycin-C was approximately 20% to 30% of the vocal fold length, and developed 4 weeks after lysis.

DISCUSSION

Surgical options for anterior glottic stenoses have included intralesional injections, endolaryngeal procedures, and open surgical procedures, including laryngofissure with laryngeal stenting.[12-17] Intralesional injections have met with limited success, especially in advanced disease, and the endolaryngeal and open procedures can be technically difficult. In addition, these latter procedures are significantly invasive and often prove debilitating to the patient in the recovery period. They commonly require an extended anesthetic exposure, inpatient hospitalization, and postoperative tracheotomy dependence.

The recent otolaryngology literature has explored topical mitomycin-C as a means of reducing granulation tissue formation and subsequent scarring in the posterior glottis[9] and the trachea,[10,11] with promising results in both areas.

This study demonstrates that a recurrent stenosis of the anterior glottis may be induced reproducibly in the canine model with the CO_2 laser. After induction of this stenosis, re-formation of an anterior glottic stenosis occurred in 100% of dogs treated with lysis alone, as compared to only a 20% recurrence rate in those lesions treated with lysis and immediate placement of topical mitomycin-C (see Figure). This method of establishing stenosis and recurrence of the stenosis complicates the experiment. However, we believe that the results from our earlier study of subglottic stenosis in a canine model validate the method used.[18] In the earlier study, we demonstrated that the stenosis is recurrent and recurs more rapidly after the first and second treatments with the laser.

In this experiment, the scar tissue was still fairly young when the mitomycin-C was applied. It is not known whether this factor played any role in the action of the mitomycin-C. Clinically, the webbing is frequently composed of long-lasting scar tissue. It might be necessary to excise this tissue and treat the more immature adhesion as it grows.

Within the field of ophthalmology, mitomycin-C is most commonly used as a 0.02% to 0.04% topical solution during such procedures as glaucoma filtration surgery, dacryocystorhinostomy, pterygium excision, and strabismus surgery.[4-8] Unlike the wide range of potentially severe side effects seen with the systemic administration of mitomycin-C, these studies have revealed largely mild local adverse reactions without significant systemic toxicity. In the present study, no local adverse tissue reactions were noted, and no systemic toxicity impairing an animal's general health was detected.

It has been our experience and the experience of others that 20% to 25% of webbing and stenosis cases do not respond to the topical application of mitomycin-C.[10,19] It may be possible to reduce that percentage with multiple treatments, as Eliashar et al[19] showed, or with injection of the mitomycin-C. Because of the toxicity of the drug, these delivery mechanisms need additional study.

Although we did not look at voice quality in this study, a related study did consider voice quality when mitomycin-C was applied to the vocal folds after the mucosa was excised.[20] In that study, mitomycin-C inhibited the regrowth of not only the collagen, but also the elastin. As a result, the videostroboscopic findings of canine vocal folds appeared worse for the vocal folds that were treated with mitomycin-C than for controls without treatment. We must therefore conclude that although mitomycin-C can help to reduce the webbing and make breathing easier, one should not expect the voice to be fully and completely restored.

CONCLUSION

In this study, we demonstrate that a recurrent stenosis of the anterior glottis may be induced reproducibly in a canine model with a CO_2 laser. In addition, application of topical mitomycin-C after lysis of an anterior glottic stenosis produces a statistically significant reduction in the rate of restenosis as compared to surgical lysis alone. We suggest this technique as a possible replacement for or adjunct to the therapies currently in use.

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Ann Otol Rhinol Laryngol 110:2001

CILIARY BEAT FREQUENCY IN CULTURED HUMAN NASAL EPITHELIAL CELLS

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This study aimed to determine the characteristics of ciliogenesis and the ciliary beat frequency (CBF) of cultured human nasal epithelial cells by means of an in vitro air-liquid interface (ALI) culture system. On the 14th, 21st, and 27th days of ALI culture, CBFs of cultured cells were measured with a video computerized analysis system, and the epithelial cell–collagen matrix complex was examined by scanning and transmission electron microscopy. Using a CBF distribution map, we calculated the proportion of ciliary beating area (CBA) on the cultured cells. On the 14th day, ciliated cells could be easily distinguished from other cells on scanning electron microscopy by their elongated cilia. Between the 14th and 27th days, the number of mature cilia increased, and after 27 days of air exposure, the cilia of each cell pointed to one direction. From the beginning of air-exposure culture until the 7th day, the number of secretory cells increased; however, from the 7th day to the 27th day, it decreased, and the number of ciliated cells increased. Total CBAs increased from the 7th day to the 21st day. The proportions of actively beating cells and the mean CBFs of beating cells among cultured epithelial cells increased with culture time. On the 21st day, the mean CBF of the cultured cells was similar to that of nasal ciliated cells in biopsy specimens (10.9 ± 0.5 Hz versus 11.4 ± 1.3 Hz), but until the 27th day, the CBF of cultured cells increased significantly (13.9 ± 0.8 Hz). It is suggested that there may be some difference in CBF between nasal epithelial cells submitted to biopsy and nasal epithelial cells cultured by the ALI culture system.

KEY WORDS — air-liquid interface, ciliary beat frequency, ciliogenesis, nasal epithelial cell.

INTRODUCTION

It has been suggested that the nasal mucociliary transport mechanism could be impaired in patients with chronic sinusitis.[1,2] Mucociliary clearance is regulated by ciliary beating, the rheological properties of the mucus, and the interaction between these two factors. Measurement of ciliary beat frequency (CBF) is of paramount importance in the understanding of the mucociliary transport system; an objective, quantitative method that can analyze CBF is desirable for clarifying the pathophysiology of respiratory disease. We developed a video computerized analysis system that can measure the CBFs of single ciliated cells and provide spatial distribution mapping between them.[3,4]

Tissue culture techniques are often used to study the pathogenesis of respiratory mucosal disease, as well as the effects of drugs or environmental pollutants on CBF. Several nasal epithelial cell culture systems have been devised to study the mechanisms of differentiation, ciliogenesis, and ciliary motility.[5-7] Respiratory tissues in organ, explant, and monolayer cultures are usually immersed in culture medium and therefore lack an air-mucosa interface. Nasal tissue, however, is constantly exposed to air and various ambient materials. An air-liquid interface (ALI) culture with tracheobronchial cells has been designed to simulate the physiologic conditions in vitro more closely.[8-11] An ALI culture system of human nasal epithelial cells may also closely simulate the nasal mucosa for study of nasal mucosal disease and ciliary motility. It is, however, important to note that cultured in vitro epithelial cells may lose some of the characteristics that would be found on biopsy of epithelial cells. Basic CBF data on cultured nasal epithelial cells and nasal epithelial cells submitted to biopsy are a prerequisite for the proper interpretation of experimental results derived from cultured cells. Observation of changes in the surface morphology of cultured cells and in the characteristics of CBF according to the culture period may be important for studies that use culture techniques. It has been reported that ciliogenesis in the sequential monolayer-suspension culture results in quantitatively normal ciliary activity, with low variability.[12]

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Using a video computerized analysis system, we determined the characteristics of an in vitro ALI culture system by observing ciliogenesis and changes in ciliary function in cultured nasal epithelial cells compared with nasal epithelial cells submitted to biopsy. We observed changes in surface morphology with respect to CBF in order to investigate the relationship between morphological and functional changes of cilia in cultured nasal epithelial cells.

MATERIALS AND METHODS

Primary Nasal Epithelial Cell Culture. Human nasal epithelial cells were obtained from nasal polyps and sinus mucosa of 28 patients who had chronic sinusitis with nasal polyps. The tissues were cut into small pieces and incubated in Dulbecco's modified Eagle's medium and Ham's nutrient F12 (DMEM-F12; Gibco BRL, Grand Island, New York) supplemented with antibiotic-antimycotic solution (Gibco BRL; 50 IU/mL of penicillin, 50 µg/mL of streptomycin, and 1 µg/mL of amphotericin B) for 2 hours. The tissues were treated with DMEM-F12 mixed with 0.1% protease (type XIV, Sigma Chemical, St Louis, Missouri) and antibiotics for 2 hours while being stirred by a magnetic bar. The enzymatically dissociated cell suspension was resuspended in DMEM-F12 containing 10% Nu-Serum (Collaborative Research Co, Bedford, Massachusetts), 10 ng/mL choleratoxin (Sigma), and 10^{-7} mol/L retinoic acid (Sigma). To isolate fibroblasts by differential attachment to a plastic dish, we preplated the cell suspensions on a 100-mm plastic dish for 1½ hours at 37°C. Suspended epithelial cells were collected and seeded at 2×10^5 cells per 30-mm well (Millipore Co, Bedford) supported by a collagen matrix, which was composed in a 6:1:3 ratio of collagen (rat tail type I collagen; Becton Dickinson Inc, Bedford), sodium bicarbonate, and culture medium, respectively. Lethally irradiated 3T3 mouse fibroblasts were used as a feeder layer; these had been cultivated in 3 mL of collagen matrix with DMEM containing choleratoxin and retinoic acid for 24 hours before the epithelial cells were plated. The millipore was placed on a 6-well plate and filled both inside and out with culture medium consisting of DMEM-F12 supplemented with Nu-Serum, choleratoxin, and retinoic acid. The millipore has 3-µm pores in the membrane. For submerged cultures, collagen on the millipore was used as a substratum, and mouse 3T3 fibroblast cells were used as a feeder layer. Epithelial cells were inoculated apically on collagen, with 2 million cells per millipore. They were grown under conditions of submersion, and when confluence was reached, the culture system was changed to an ALI by adjusting the level of the culture medium. The medium outside the millipore reached the collagen substratum, and inside medium was not supplied; under these conditions, nutrition was supplied by the outer medium through the millipore. The culture medium was renewed every day. On the 7th, 14th, 21st, and 27th days, the CBF was measured with a video computerized analysis system. The epithelial cell–collagen matrix complex was processed for scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

Electron Microscopic Examination. For SEM, specimens were fixed in 2.5% glutaraldehyde and rinsed with 0.1 mol/L phosphate-buffered saline solution (PBS). They were postfixed in 1% osmium tetroxide and washed with 0.1% tannic acid. This was followed by dehydration through serial ethanol solutions and finally in a critical point dryer (SCP-2; Hitachi Co Ltd, Tokyo, Japan). They were then coated with gold in a sputter coater at an acceleration voltage of 2.5 kV, and the length of the cilia, the number of microvilli, and the number of abnormal cilia were examined through a Hitachi H-570 scanning electron microscope.

For TEM, specimens were fixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated in serial ethanol solutions, and embedded in Epon. Randomly selected ultrathin sections through the central part of each specimen, typically consisting of 70 to 100 cells, were examined through a Hitachi H-600 transmission electron microscope. Ciliated cells were identified by the presence of basal bodies, and secretory cells by the absence of basal bodies and the presence of large secretory vesicles in their cytoplasm.[13] In TEM, ciliated cells and secretory cells were calculated as the number of cells per millimeter of surface epithelium.

Measurement of Ciliary Beat Frequency and Distribution Mapping of Beating Cells in Cultured Nasal Epithelial Cells. The CBFs were measured with a video computerized analysis system described previously.[4] The system can measure CBF variation between cells and show its distribution among cells in an acquired field.[3] Microscopic ciliary images are transformed into digitized gray ones through an image-grabber inside a personal computer, and from these, the system extracts signals based on an image-subtraction algorithm and processed through a power spectrum analysis using fast Fourier transform (FFT). By means of the FFT power spectrum, the maximum peak frequencies for the entire digitized field can be detected as the CBF in each partitioned block. Using these CBFs, we composed distribution maps of various resolutions to show the spatial distribution

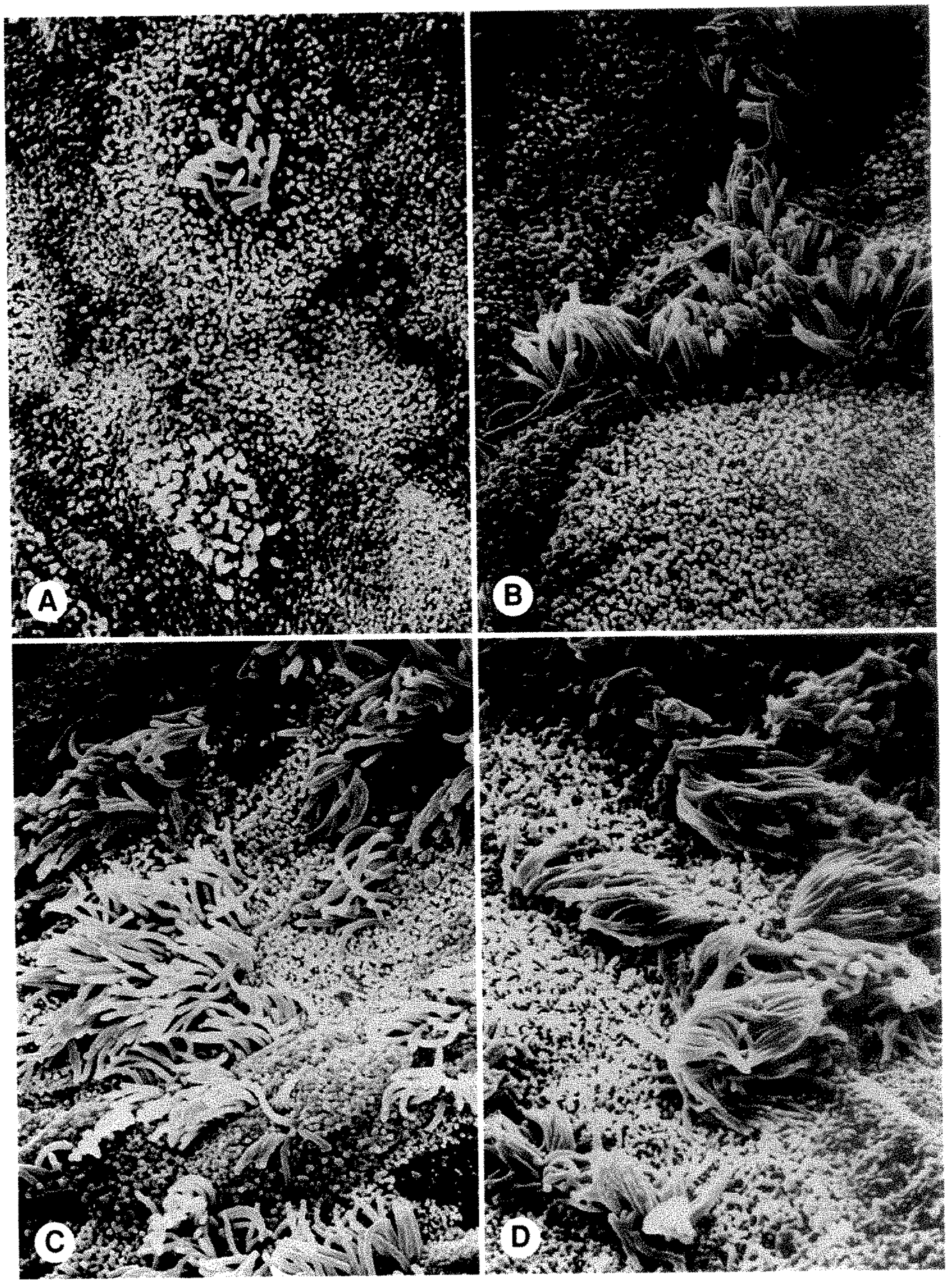

Fig 1. Scanning electron microscopic findings. **A)** After 7 days of air exposure. Several cells have cilialike protrusions. **B)** After 14 days of air exposure. Elongated structures are more pronounced, and number of microvilli per cell is greater than on 7th day. **C)** After 21 days of air exposure. Number of matured ciliated cells is increased; however, even in single cell, ciliary direction is not uniform. **D)** After 27 days of air exposure. In each cell, cilia point in same direction.

of CBFs through cells and in a single cell.

The frequency of 0 Hz was represented as a palette of blue, 7.5 Hz as green, and 15 Hz as red. Intermediate frequencies from 0 to 7.5 Hz were linearly interpolated by decreasing the blue value of the palette and increasing the green value, and frequencies from 7.5 to 15 Hz by decreasing the green value and increasing the red value. The CBF distribution map in various block sizes clearly showed that the spatial distribution of ciliary beating cells among cultured nasal epithelial cells could be easily seen according to their CBFs. Using this map, we calculated the proportion of beating areas in these epithelial cells and divided them arbitrarily into 3 categories: slowly beating area (mean CBF less than 7.0 Hz), moderately beating area (7 to 10 Hz), and actively beating area (more than 10 Hz). In a specimen used for this study, the average value of 4 areas with more than 10 actively beating cells in each cultured specimen was taken as the CBF. The CBF of original nasal epithelial cells was measured as described previously.[4] The data are presented as the average of 4 experiments.

Statistical Methods. The values are means ± SD. Mean CBFs in biopsy specimens and cultured specimens were analyzed with the Wilcoxon's rank sum test at a statistical significance of .05.

RESULTS

The cultured epithelial cells stained with anticytokeratin antibodies; the pattern of cytokine expres-

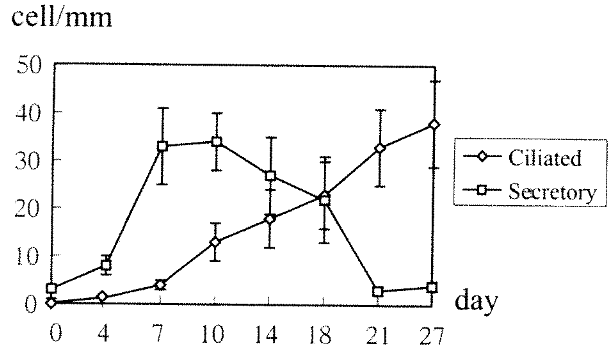

Fig 2. Changes in proportion of ciliated and secretory cells during culture period.

sion indicated that cultured cells were nasal epithelial cells. Four days after exposure to air, the cell surface was scattered with microvilli; the number of microvilli per cell was higher on the fourth day of air exposure than on the first day. On the 7th day, several cells showed elongated structures resembling ciliary protrusions (Fig 1A), and until the 10th day, the number of such cells increased. The protrusions were longer on the 14th day of air exposure than on the 7th day (Fig 1B). The elongated structures resembled mature cilia and were clearly distinguished from microvilli. On the 21st day of air exposure, the number of mature ciliated cells had increased and accounted for 25% of cells; the ciliary direction, however, was not uniform, probably because of random movement of cilia (Fig 1C). On the 27th day of air exposure, the number of mature cilia had slightly increased, and in the most mature ciliated cells, the cilia pointed in one direction (Fig 1D).

By TEM, ciliated and secretory cells were counted as the number of cells per millimeter of surface epithelium. At the beginning of the air exposure culture, secretory cells increased until the 7th day; however, the number decreased, while the number of ciliated cells increased, until the 27th day (Fig 2).

The CBF of original nasal cells was 10.9 ± 0.5 Hz. The number of actively beating cells (from green to red) in the ALI culture system increased with the culture period. On the 7th day of ALI culture, ciliary beating was recognized in a small proportion of cultured cells; total ciliary beating areas increased from the 7th day to the 21st day (see Table). The actively beating area on a CBF distribution map was smaller than the area of mature cilia seen on SEM, and even on the 27th day, the CBFs were not the same in all cultured areas. The proportions of actively beating cells and the mean CBF of beating cells in cultured epithelial cells increased from the 14th day to the 27th day of ALI culture. From the 21st day to the 27th day, the proportions of actively beating cells did not change, whereas the mean CBF increased from 11.4 ± 1.3 Hz to 13.9 ± 0.8 Hz. The CBF on the 27th day was significantly higher than the CBF of the epithelial cells submitted to biopsy ($p < .05$).

DISCUSSION

In this study, nasal cell culture using an ALI system easily yielded ciliated cells, and coordinated ciliary beating was observed. These characteristics are important for the study of mucociliary clearance. Morphological changes of cilia, increased CBF, and increased ciliary beating area (CBA; the proportion of ciliated cells on the CBF distribution map) were also demonstrated over the culture period. In 1994, Hanamure et al[7] developed a floating culture system that effectively induces the differentiation of cultured human nasal epithelial cells into ciliated cells that morphologically resemble in vivo ciliated epithelium. A disadvantage of this method, however, is that because the collagen matrix thins with time, its long-term maintenance is difficult. The matrix shrinks because of marginal curling of collagen after it is floated. Because the cultured cells become smaller with time, it is difficult to perform simultaneous morphological and molecular studies. To solve the problem of collagen curling and to obtain sufficient cultured cells, we developed the ALI raft culture system. Monolayer culture has been used to study the CBF of nasal epithelial cells[5]; however, because the CBF and CBA in this culture system continuously decrease with time, long-term maintenance may be difficult.[11] Human respiratory cells cultured in monolayer cell culture gradually lose their cuboidal shape and cilia with increasing culture time,[11] whereas ciliated cells in suspension cultures can preserve their respiratory-type cuboidal morphology for several months without degeneration of the ciliary beat.[14] Suspension culture of nasal epithelial cells is very effective and useful

PROPORTION OF CILIARY BEATING CELLS IN CULTURED HUMAN NASAL EPITHELIAL CELLS WITH CULTURE TIME

Culture Time	*N*	*Slowly Beating Area*	*Moderately Beating Area*	*Actively Beating Area*	*Total Beating Area*
Day 7	4	8.4 ± 1.3	3.7 ± 1.1	2.5 ± 1.3	14.6 ± 1.2
Day 14	4	24.5 ± 3.4	5.9 ± 2.1	6.4 ± 2.8	36.8 ± 3.1
Day 21	4	12.8 ± 2.9	27.2 ± 5.3	16.3 ± 2.6	56.3 ± 3.8
Day 27	4	11.1 ± 2.7	20.2 ± 4.2	23.8 ± 4.6	55.1 ± 4.2

Data are percentages (mean ± SD).

for ciliogenesis and measurement of CBF of the cultured cells.[12] The epithelium generated on type I collagen gel substratum tends to be somewhat taller, and the cells are columnar. Significantly more ciliated cells develop than when substratum with no collagen gel is used.[9] These reports suggest that for the maintenance of ciliary function, the cuboidal or columnar form is important. In contrast to the findings of a previous report,[7] ciliogenesis in ALI culture in this study was slightly slower than that in floating culture.[5] Because cultured cells in floating culture are likely to be tall or cuboidal ones because of the mechanical force released by the shrinkage of collagen matrix, ciliogenesis may occur earlier in floating culture than in ALI culture.

The finding of an early increase in the number of secretory cells is in agreement with findings of other reports.[7,8] However, it is unclear why ciliated cells increase, while secretory cells decrease, in late stages of culture. It has been suggested that ciliated cells do not divide and thus play no important role in the regeneration of mucociliary epithelium.[15] In addition, it has been demonstrated that both basal and secretory cells are able to regenerate a mucociliary phenotype.[16,17] From the reports and our results, it appears that attachment of preferred ciliated cell precursors is not the main factor in ciliogenesis in culture.

The CBF can be studied in more detail by the video computerized CBF distribution map than by previously used photodetector methods. From the color-coded CBF distribution map, differences in CBFs of a single cell in the examined fields are easily measured and distinguished. In this study, the CBA increased as the culture time elapsed. On the CBF distribution map or SEM, the CBA was shown as a clustered rather than a scattered area — a finding suggesting that an intracellular relationship or cell-to-cell contact of ciliated cells may induce ciliogenesis on nonciliated cells in culture.

The CBF and the proportion of actively beating area increased with time. The CBF of an actively beating area on the 21st day was similar to that of epithelial cells submitted to biopsy, and on the 27th day, it was slightly higher. In organ culture incorporating an air interface, the CBF decreased from 11.6 ± 0.2 Hz on the first day to 10.6 ± 0.3 Hz after 20 days.[13] In another study using a floating culture,[18] numerous short cilia with a high CBF (17.8 ± 2.5 Hz) were observed on the 8th day, and the mean CBF was highest on the 10th day (19.6 ± 3.9 Hz), reaching 17.4 ± 2.5 Hz on the 14th day. In this study, numerous short cilia were observed on SEM between the 7th day and the 14th day. Most of the beating cells, however, had a low CBF, although a small CBA had a high CBF (red zone). The difference may result from different culture techniques and the different CBF measuring systems. Morphologically immature, short cilia may beat in both culture systems, although they may have little effect on mucociliary transport.[18] In this study, the cilia of each cell were rearranged in one direction — a finding that may be indicative of synchronous ciliary movement — and CBF increased with increasing culture time from the 14th day to the 27th day. This finding suggests that the ciliary beat may gradually synchronize after the elongation and maturation of cilia and that synchronized ciliary beating may increase the CBF and effectively act on mucociliary transport. However, more functional tests are needed for the exact evaluation of synchronized movement.

We also noted that the actively beating area on the CBF distribution map, as seen on SEM, was smaller than the area of mature cilia, and that even on the 27th day, the CBFs were not the same in all cultured areas. This finding suggests that although the area of mature cilia seen on SEM increased with culture time, there were fewer functioning cilia than morphologically mature cilia.

In conclusion, for studying the regulation of ciliogenesis, as well as ciliary movement, an in vitro model of human nasal epithelial cells cultured with air exposure and a CBF distribution mapping system may be useful. There may be some difference in CBF between nasal epithelial cells submitted to biopsy and cultured nasal epithelial cells in an ALI culture system.

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THREE-DIMENSIONAL ANALYSIS OF MORPHOLOGICAL ASPECTS OF THE HUMAN SACCULAR MACULA

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The 3-dimensional shape of the human saccular macula and its orientation in the skull were quantitated in this study. The semicircular canals and saccular maculae were reconstructed 3-dimensionally on a computer from 3 human temporal bones. The 380 to 522 triangles in the entire area of the saccular macula were made by drawing lines between 2 adjacent points every 100-µm width of the saccular macula in each section. The angles between each triangle and each estimated standard axis in the skull obtained were calculated. This information will provide standard data regarding the 3-dimensional morphological aspects of the saccular macula for further investigations of the function of the sacculus. It was determined that the 3-dimensional shape of the saccular macula was not a plane, but was a curved surface like that of an ellipsoid. It is thought that this shape is necessary in order for the saccular macula to detect wide-range linear acceleration.

KEY WORDS — otolithic organ, saccular macula, three-dimensional analysis, three-dimensional reconstruction, vestibular end organ.

INTRODUCTION

The otolithic organs are known as the specific organs that can detect the position and direction of the head and the effect of linear acceleration, including gravity, on the head. These organs consist of the otolithic membranes, the hair cells, the supporting cells, and the synapses. The otolithic membrane contains crystals of calcium carbonate (specific gravity, 2.71[1]), which are heavier than the endolymph (specific gravity, 1.003) existing in the otolithic membranes. The otolithic membranes slide over the cilia of the hair cells when linear acceleration is applied or the gravity field is changed. The direction of linear acceleration that stimulates the otolithic organs effectively is tangential to the plane of the otolithic macula.[2,3]

In the investigation of the function of the vestibu-

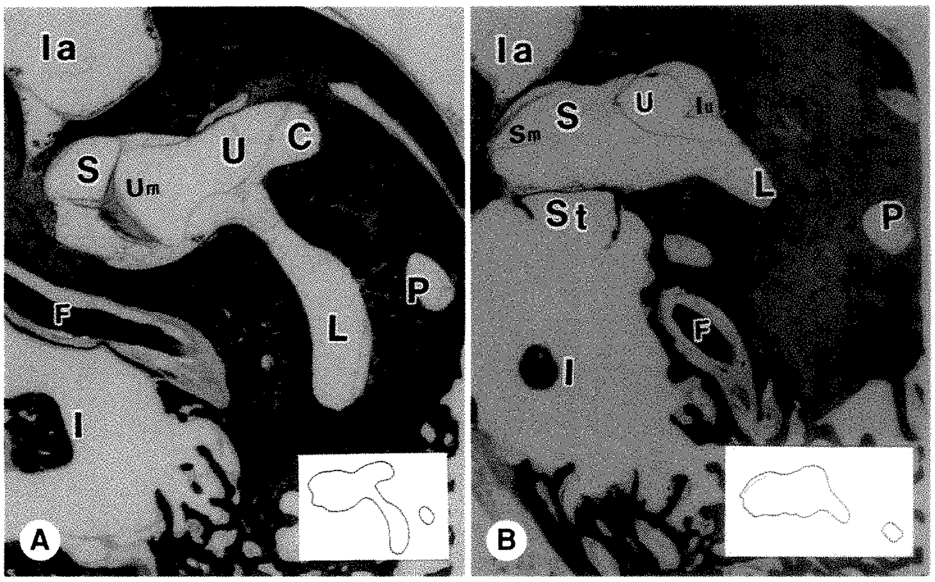

Fig 1. Horizontal histologic sections (20 µm) at levels of **A**) utricular macula and **B**) saccular macula of human temporal bone. Contour of bony labyrinth (and in B, of saccular macula) was drawn precisely as shown at bottom right corner. Ia — internal auditory meatus, S — sacculus, U — utriculus, Um — utricular macula, C — common crus, L — lateral semicircular canal, P — posterior semicircular canal, F — facial nerve, I — incus, Iu — inferior utricular crest, St — stapes.

From the Departments of Otorhinolaryngology (Naganuma, Tokumasu, Okamoto, Hashimoto) and Anatomy (Yamashina), Kitasato University School of Medicine, Kanagawa, Japan. This study was funded in part by the National Institutes of Health/National Institute on Deafness and Other Communication Disorders (grant DC-00589) and The Japan Foundation for Aging and Health.

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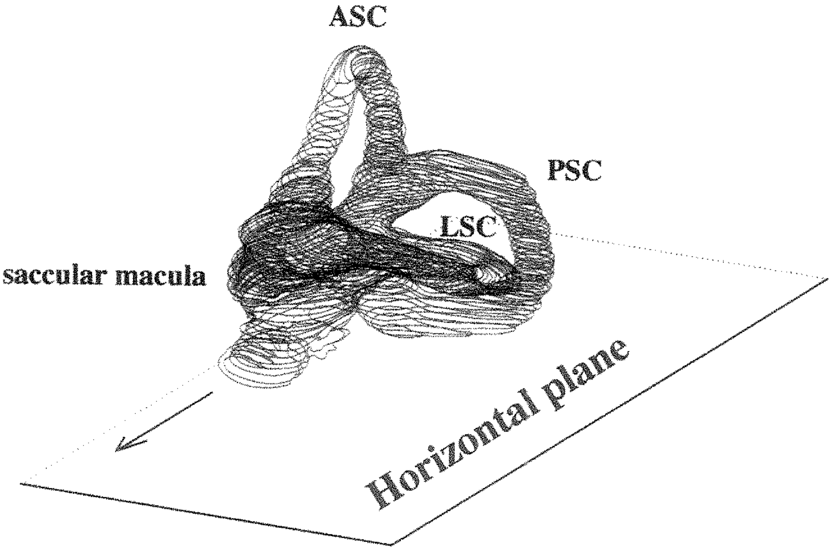


Fig 2. Structures of left bony labyrinth, including saccular macula and reference holes, were drawn accurately; these drawings were scanned into computer (Apple Macintosh), and data points were digitized with digitizer. Three-dimensional reconstruction of these temporal bone sections was made with 3-dimensional CAD/CAM (computer-aided design/computer-aided manufacturing) software with proper alignment of reference holes. ASC — anterior semicircular canal, LSC — lateral semicircular canal, PSC — posterior semicircular canal.

lar organs, especially the otolithic end organs, it is important to consider the morphological aspects of these end organs relative to the stereotaxic planes of the head.

Clinically, however, although a function test for the semicircular canals is usually carried out, a test for the otolithic organs is not. This is because methods of stimulating the otolithic organs effectively have not been established. In order to establish such a method, it was thought that accurately measuring the 3-dimensional (3-D) shape and direction of the otolithic maculae in the skull was a necessary step.

In this present study, to establish standard data on the 3-D morphological aspects of the human saccular macula (macula sacculi), the angles between the human saccular macula and the standard axes in the

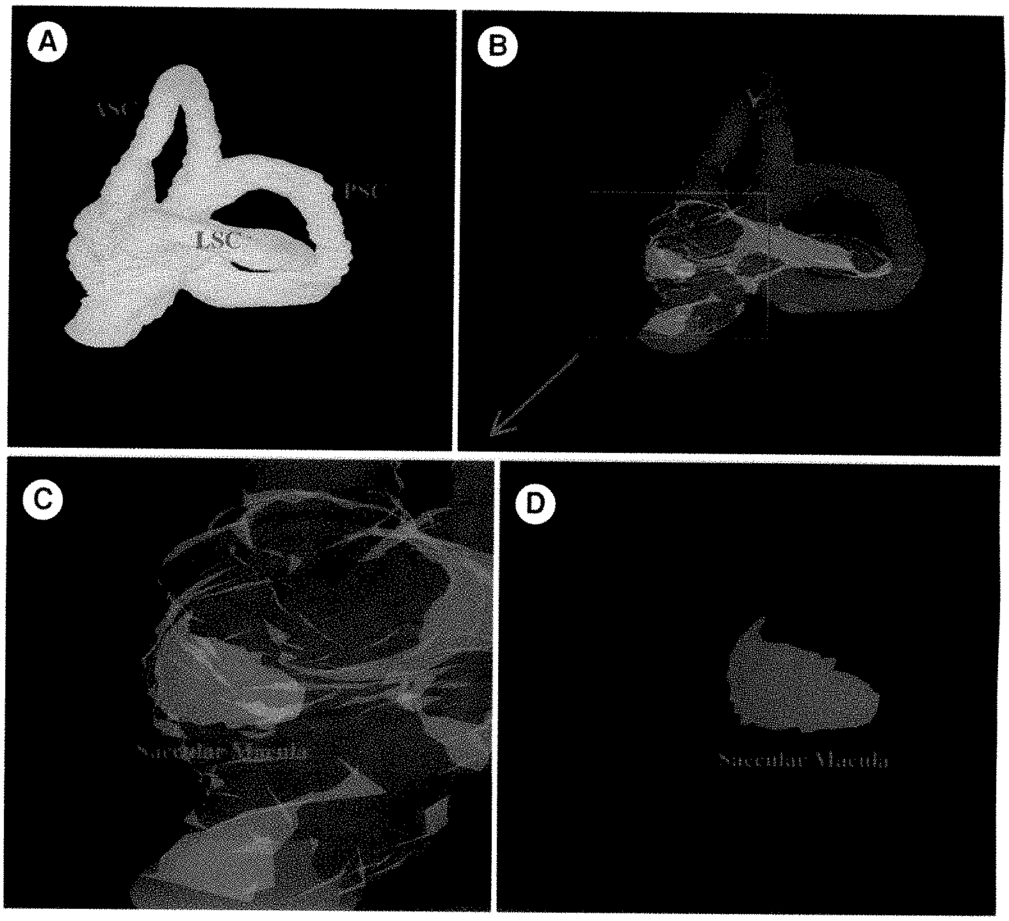


Fig 3. Reconstruction of specimen shown in Fig 1, made with rendering software. **A)** Structures of left bony labyrinth, ASC, LSC, and PSC. **B)** Structures of same bony labyrinth, which was 90% permeable. Saccular macula could be seen. **C)** Saccular macula was magnified. **D)** It was demonstrated that saccular macula was not plane, but curved surface similar to surface of ellipsoid.

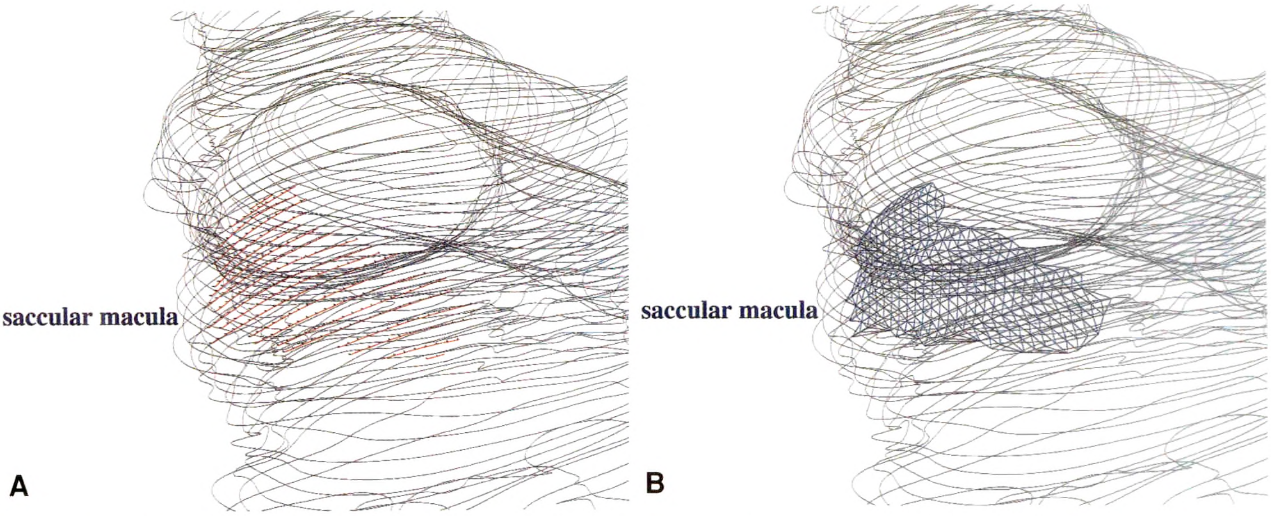


Fig 4. Analysis of morphology. **A)** Saccular macula of each layer was divided into multiple segments of 100 µm per segment and marked. **B)** Microtriangles were drawn with marks described in Methods section, in which sum total of length of sides of triangles was shortest. Ultimately, curved surface of saccular macula was divided into microtriangles.

skull were quantitated.

MATERIALS AND METHODS

We used 3 human temporal bones in which no malformations of the ear were found, from a consecutive temporal bone collection at Wake Forest University School of Medicine (Winston-Salem, North Carolina). Temporal bone 1 was from the left ear, temporal bone 2 from the right ear, and temporal bone 3 from the left ear. The temporal bones had been removed at the time of autopsy, prepared for histologic evaluation, embedded in celloidin, and serially sectioned at 20 µm. Before sectioning, 3 or 4 holes were drilled (with a high-speed drill press with a 0.7-mm-diameter drill bit) in the blocked temporal bone as reference points to be used for 3-D reconstruction. Every 10th section was stained with hematoxylin and eosin and mounted; but where the otolithic organs were identified, every 5th section was stained and mounted.

At first, each section of the temporal bone was evaluated under a light microscope. Then, each section was accurately enlarged by 14× magnification onto an acetate film with a projector. Structures of the bony labyrinth, including the saccular macula and reference holes, were drawn precisely; these drawings were scanned into a computer (Apple Macintosh), and data points were digitized with a digitizer (Numonics, Graphic Master II; Fig 1). A 3-D reconstruction of these temporal bone sections, shown in wire-frames in Fig 2, was made with 3-D CAD/CAM (computer-aided design/computer-aided manufacturing) software (Ashler Vellum 3-D) with proper alignment of the reference holes. Based on the wire-frame data (Fig 2), 3-D reconstructions (Fig 3) were made

AVERAGE EQUATIONS OF PLANES OF SCCs

Plane	*Equation*
Left lateral SCC	$0.365x - 0.158y - 0.905z = 0$
Left anterior SCC	$0.652x - 0.753y - 0.017z = 0$
Left posterior SCC	$0.757x + 0.561y + 0.320z = 0$
Right anterior SCC	$0.652x + 0.753y - 0.017z = 0$
Right lateral SCC	$0.365x + 0.158y - 0.905z = 0$
Right posterior SCC	$0.757x - 0.561y + 0.320z = 0$

Adapted from Blanks et al.[4]
SCC — semicircular canal.

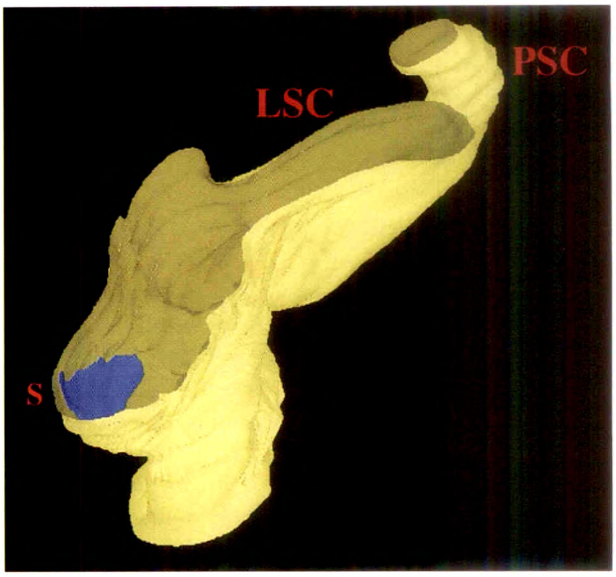


Fig 5. Position of saccular macula (S) in peripheral vestibular organ and 3-dimensional shape of saccular macula are demonstrated. Saccular macula is not plane, but curved surface like surface of ellipsoid. To approximate inside of labyrinth, we eliminated upper structures of bony labyrinth and saccular macula in this Figure. LSC — lateral semicircular canal, PSC — posterior semicircular canal.

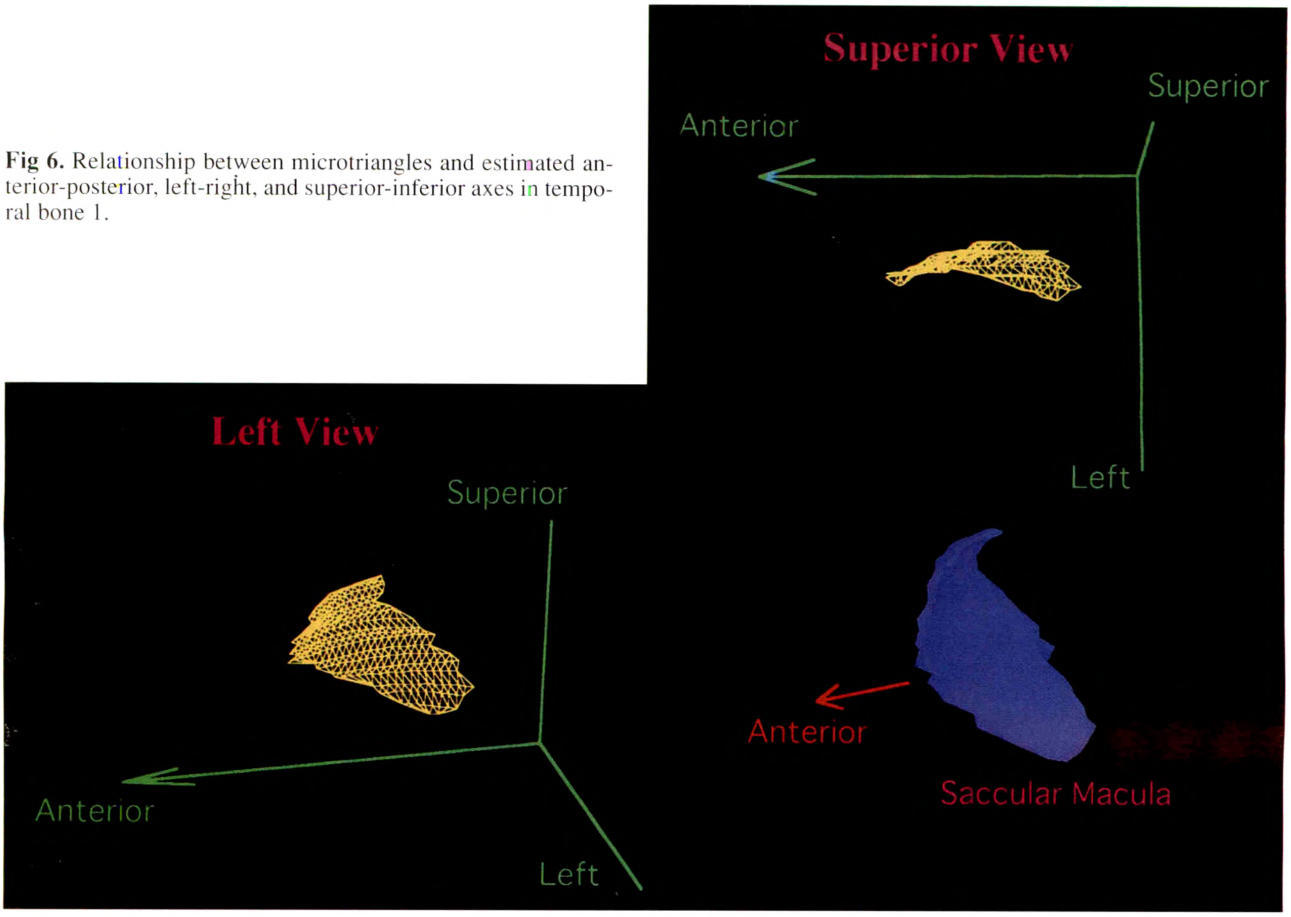


Fig 6. Relationship between microtriangles and estimated anterior-posterior, left-right, and superior-inferior axes in temporal bone 1.

with rendering software (Tri Ratock systems).

Once the 3-D reconstruction of the saccular macula was completed, analysis of the morphology of the structure was performed by the following method (Fig 4). Since the distance between the 2 layers of the temporal bone sections was 100 µm, the saccular macula of each layer was divided into multiple segments of 100 µm per segment and marked. The microtriangles were drawn by use of the marks. The drawing of each triangle was made according to the rule that the sum total of the length of the sides of the triangles was shortest to prevent bias. Ultimately, the curved surface of the saccular macula was divided into approximately 500 microtriangles. In this way, a modified finite element analysis method could be applied to determining the actual shape of the saccular macula, and the equations of the planes of these microtriangles could be determined relative to the estimated axis of the skull and the angles between the microtriangles and the estimated standard axes (the anterior-posterior [A-P], the left-right [L-R], and the superior-inferior [S-I] axes) of the skull. The equations for the estimated standard axes of the skull in the subjects' temporal bones were based on the average equations of the planes of the lateral and posterior semicircular canals developed previously on the basis of the Reid stereotaxic reference system.[4] The average equations of the planes of the semicircular canals are shown in the Table.[4]

RESULTS

The position of the saccular macula in the peripheral vestibular organ is shown in Figs 3 and 5, which demonstrate that the 3-D shape of the saccular macula was not a plane, but a curved surface like that of an ellipsoid.

The equations of each plane of the microtriangles expressing the surface contour of the saccular macula based on the Reid stereotaxic horizontal plane were determined.[4] The angles between each plane of the microtriangles and each estimated standardized axis were determined to be as follows (Figs 6-9). The relationships between the microtriangles and each estimated standardized axis are also shown in Fig 6 (temporal bone 1).

In temporal bone 1, the angles between the microtriangles and the A-P, L-R, and S-I axes ranged from 0° to 68.0° (median, 13.3°), from 0° to 90.0° (median, 57.4°), and from 0° to 71.2° (median, 23.4°), respectively (Fig 7).

In temporal bone 2, the angles between the micro-

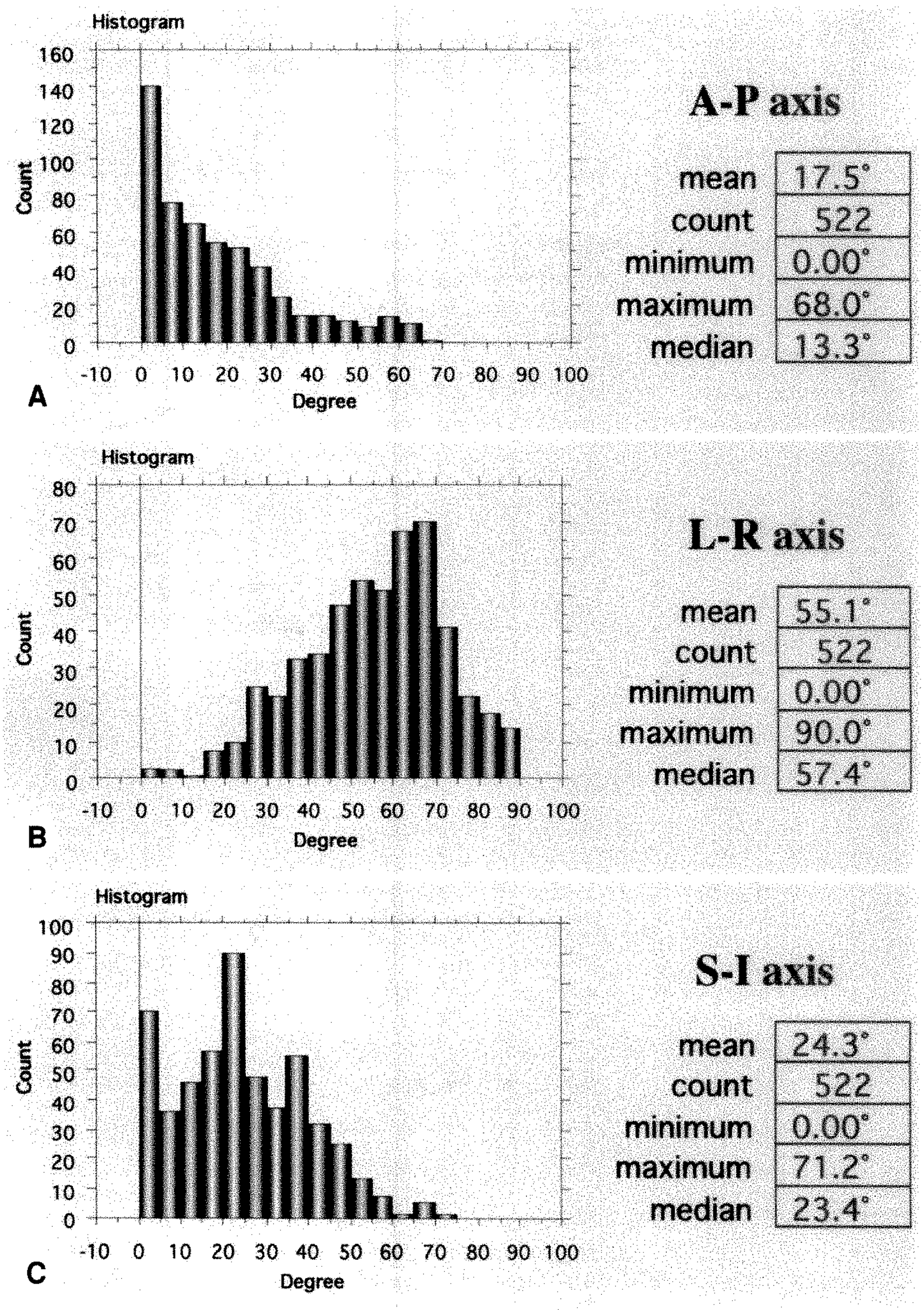


Fig 7. Angles between microtriangles and estimated standard axes for temporal bone 1. Histograms demonstrate number of microtriangles in each class of angle. **A)** Angles between each plane of microtriangles and estimated anterior-posterior (A-P) axis. Number of microtriangles that were close to parallel to A-P axis was large. **B)** Angles between each plane of microtriangles and estimated left-right (L-R) axis. Number of microtriangles that were close to parallel to L-R axis was small. **C)** Angles between each plane of microtriangles and estimated superior-inferior (S-I) axis. Number of microtriangles that were close to parallel to S-I axis was large.

triangles and the A-P, L-R, and S-I axes ranged from 0.031° to 76.1° (median, 24.0°), from 0.08° to 88.0° (median, 58.1°), and from 0.09° to 76.1° (median, 13.1°), respectively (Fig 8).

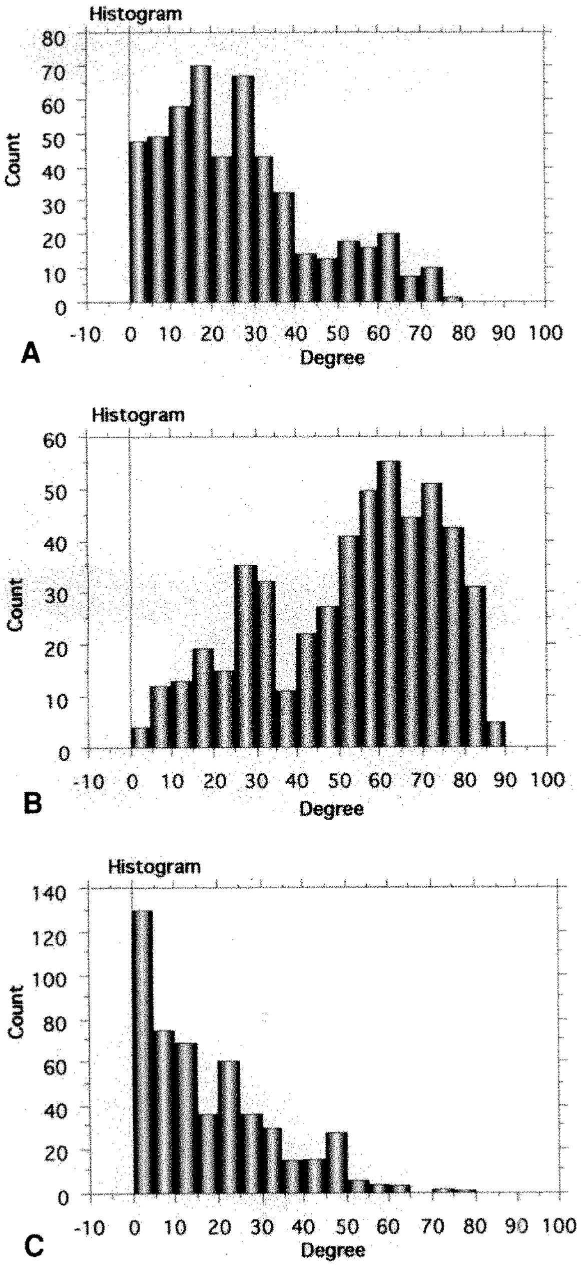
In temporal bone 3, the angles between the microtriangles and the A-P, L-R, and S-I axes ranged from 0.04° to 70.5° (median, 23.8°), from 0.20° to 89.5° (median, 41.7°), and from 0.08° to 76.0° (median, 29.5°), respectively (Fig 9).

The average areas of the microtriangles were 0.0057 mm^2 (SD, 0.0001 mm^2), 0.0056 mm^2 (SD, 0.00083 mm^2), and 0.0056 mm^2 (SD, 0.00064 mm^2) in temporal bones 1, 2, and 3 respectively, and in the same temporal bone there were no major differences in the areas of each of the microtriangles.

DISCUSSION

In this study, the standard axes in the skull were developed on the basis of the average equations of the lateral and posterior semicircular canal planes in the Reid stereotaxic coordinate system.[4] The reliability of the standard axes estimated in the 3 temporal bones in this study ranged from 3.7° to 8.2° (95% confidence limits of the angles between the semicircular canal planes and the standard planes reported[4]).

The 3-D reconstructions and the measurements of



A-P axis

mean	26.5°
count	508
minimum	0.031°
maximum	76.1°
median	24.0°

L-R axis

mean	52.9°
count	508
minimum	0.082°
maximum	88.0°
median	58.1°

S-I axis

mean	17.8°
count	508
minimum	0.087°
maximum	76.1°
median	13.1°

Fig 8. Angles between microtriangles and estimated standard axes for temporal bone 2. Histograms demonstrate number of microtriangles in each class of angle. **A)** Angles between each plane of microtriangles and estimated A-P axis. Number of microtriangles that were close to parallel to A-P axis was large. **B)** Angles between each plane of microtriangles and estimated L-R axis. Number of microtriangles that were close to parallel to L-R axis was small. **C)** Angles between each plane of microtriangles and estimated S-I axis. Number of microtriangles that were close to parallel to S-I axis was large.

the angles were made as accurately as possible, as described in the Methods section. In the histological preparations, the contractions of the otolithic maculae and membranous labyrinth were thought to be as follows. The contraction of the otolithic maculae occurred equally in all directions. The 3-D shape of the otolithic maculae was maintained, as well as the angles between the microtriangles expressing the surface contour of the saccular macula, and the estimated standard axes in the skull were not influenced.

The otolithic organs detect linear acceleration and gravity. Although we know that the otolithic membrane slides over the cilia of the hair cells embedded in the otolithic maculae[2,3] when the head is subjected to linear acceleration, we do not know the exact direction of movement of the otolithic membrane required to detect linear acceleration as perceived by the otolithic system. It is necessary to determine the actual direction of movement of the otolithic membrane in the human skull (as related to the 3-D shape of the macula) to create a functional test of the otolithic organs.

It was thought that the otolithic macula was stimulated effectively in response to linear acceleration in a direction tangential to its plane. In the case of the human saccular macula, are linear accelerations in the directions of A-P, L-R, and S-I effective stimulation? In the microtriangles expressing the surface

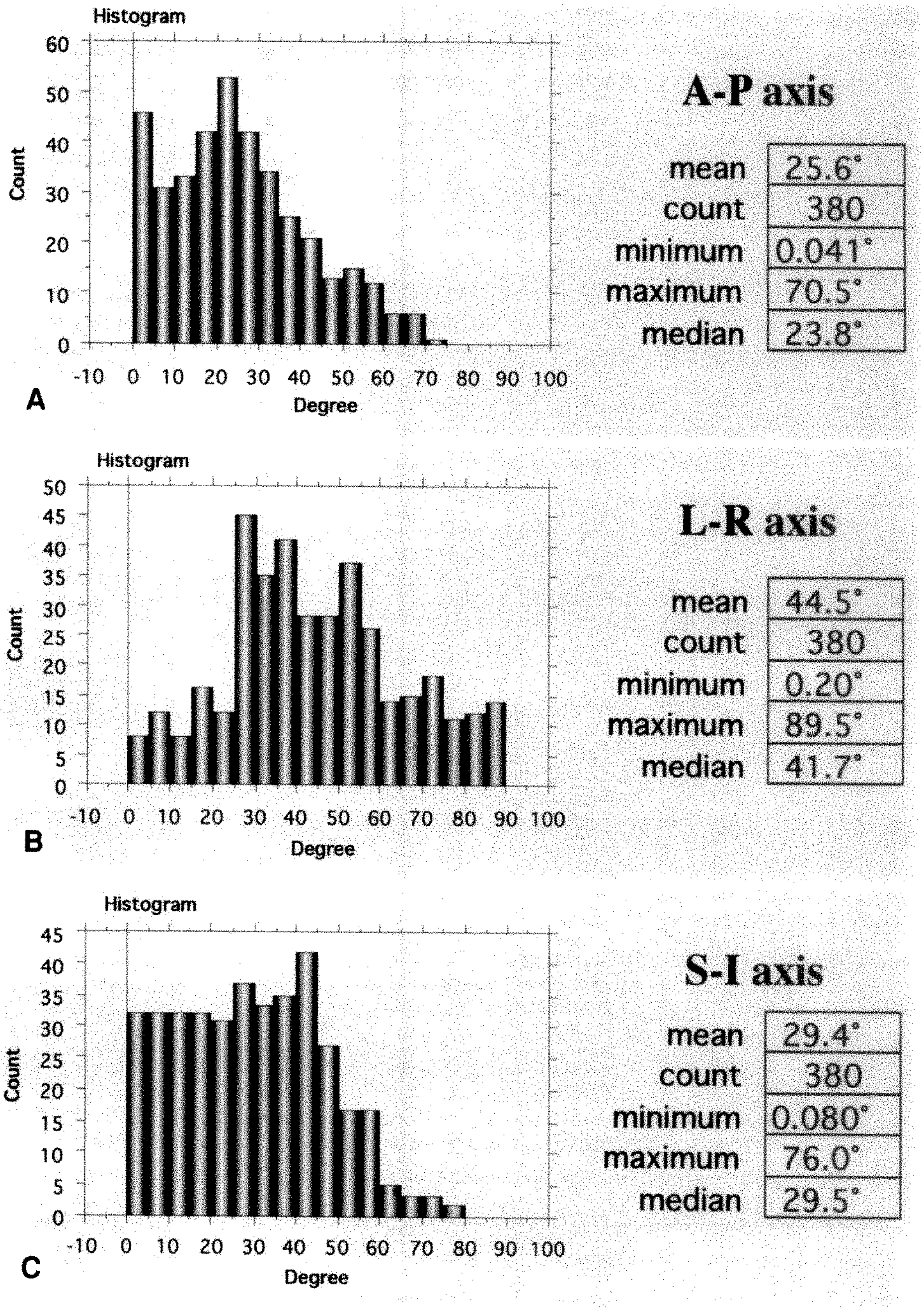


Fig 9. Angles between microtriangles and estimated standard axes for temporal bone 3. Histograms demonstrate number of microtriangles in each class of angle. **A)** Angles between each plane of microtriangles and estimated A-P axis. Number of microtriangles that were close to parallel to A-P axis was large. **B)** Angles between each plane of microtriangles and estimated L-R axis. Number of microtriangles that were close to parallel to L-R axis was small. **C)** Angles between each plane of microtriangles and estimated S-I axis. Number of microtriangles that were close to parallel to S-I axis was large.

contour of the saccular macula, how many microtriangles are close to parallel to the A-P, L-R, and S-I axes? It seems that the number of microtriangles that were close to parallel to the A-P and S-I axes was large, and the number that were close to parallel to the L-R axis was small. Since no major differences were detected in the area of any of the microtriangles, it was thought that linear acceleration in the A-P and S-I directions could stimulate the saccular macula effectively, and that linear acceleration in the direction of the L-R axis could not stimulate it effectively.

Thus far, the shape of the otolithic maculae has been investigated in only a few reports.[5-7] In these reports, the otolithic maculae were recognized as a plane or the constitution of a few planes. In both our previous[8] and present studies, it was confirmed that the 3-D shape of the human saccular macula was a curved surface similar to the surface of an ellipsoid. It is thought that there exists a relatively wide range in the direction of linear accelerations that stimulate the saccular macula effectively (Figs 3 and 5).

Within the otolithic membrane is the striola, which is shaped like a snowdrift. The striola is identifiable as a groove in the utricular macula, and in the saccular macula it appears as a ridge. In the utricular macula, the kinocilia of the hair cells are located toward the striola, while in the saccular macula, the kinocilia are located away from the striola. Although it is known that the curved striola in the saccular macula can detect widely varied directions of linear acceleration, it is possible that the 3-D morphological aspects have some important function in detecting a wide range of directions of linear acceleration.

The parallel swing test[9,10] and the sinusoidal linear acceleration test,[11,12] which induce compensatory eye movements with linear acceleration, were used to study the otolithic organ function; however, methods for stimulating the otolithic organs effectively have not been established. Neither have methods for stimulating each otolithic organ separately been described.

It is possible that the standard data regarding the 3-D shape of the human saccular macula that are reported in the present study can be utilized in the development of a new testing method for stimulating the otolithic organs effectively.

CONCLUSION

In this study, 3 normal temporal bones were utilized to develop standard data on the 3-D shape of the human saccular macula. The angles between each plane of the microtriangles and each estimated standardized axis were determined.

It was confirmed that the 3-D shape of the human saccular macula is a curved surface similar to the surface of an ellipsoid. It is thought that a relatively wide range exists in the directions of linear acceleration that stimulate the saccular macula effectively. Figure 6 (temporal bone 1) shows the relationships between the microtriangles and each estimated standardized axis. These results may further our understanding of how the saccular macula responds to linear acceleration applied to it and aid in the development of a new functional test for the otolithic organs.

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FACIAL NERVE SCHWANNOMA: SURGICAL EXCISION VERSUS CONSERVATIVE MANAGEMENT

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Facial nerve schwannomas are slow-growing tumors that may involve any part of the facial nerve. When they present with moderate to total facial palsy, complete resection is clearly indicated. However, in cases with mild or no facial dysfunction, the best course of treatment is less obvious. A series of 22 patients with facial nerve schwannoma is presented, of whom 12 underwent definitive excision and 10 were managed more conservatively. The best postoperative facial function in the group who had tumor removal was a House-Brackmann grade III, while 8 of the conservatively treated group had normal facial function up to 10 years after presentation. As well, no significant tumor growth was noted on serial radiologic imaging of those being observed. Delaying surgical resection of facial nerve schwannomas may allow patients to retain normal facial function indefinitely.

KEY WORDS — conservative management, facial nerve schwannoma, facial paralysis, surgical excision.

INTRODUCTION

Complete excision of facial nerve schwannomas almost invariably results in an initial total facial paralysis and permanent partial weakness. Clearly, this is a source of great distress to the patient, especially in cases in which facial function was normal before the operation. In addition, these tumors can mimic acoustic neuromas,[1,2] so that the true state of affairs is not obvious until surgery is carried out.

Facial nerve schwannomas are slow-growing tumors.[3,4] Delaying surgical excision is appropriate in those patients with minimal or no facial weakness. Treatment by observation and serial radiologic examination only or by tumor decompression or debulking may put off the onset of facial paralysis indefinitely.

A series of patients with facial nerve schwannoma is reviewed. We compare the outcomes of those who underwent definitive excision and those who were managed conservatively. Overall, follow-up facial nerve function is significantly better in the latter group than in the former.

MATERIALS AND METHODS

The charts of 22 patients with facial nerve schwannoma were retrospectively reviewed. All of the patients were assessed and treated by the senior author (P.F.) between 1982 and 2000. There were 10 female patients and 12 male patients. They ranged in age from 3 to 64 years.

Twelve patients underwent definitive excision of their tumors, while 10 were followed by observation and serial imaging after surgical exploration, tumor decompression or debulking, or no surgical intervention. Surgical excision was carried out by either the transmastoid approach or the translabyrinthine approach, depending on the location and extent of the tumor. Surgical exploration or tumor debulking was performed through the retrosigmoid route, while the translabyrinthine approach was used in 3 cases of wide tumor decompression.

In the group being observed, follow-up was continued to at least 3 years in 5 patients, with either magnetic resonance imaging or a high-resolution computed tomography scan repeated at 1 and 3 years. For 1 patient (case 22), regular follow-up and serial radiologic imaging were not begun until 7 years after the initial presentation. Two patients were lost to follow-up before 2 years, while 3 patients have not yet reached that duration.

The preoperative and postoperative facial nerve functions were assessed according to the House-Brackmann grading system.[5]

RESULTS

The major presenting symptoms, facial function at presentation, and facial nerve segments involved by tumor for all 22 cases are summarized in Tables 1[5] and 2.[5] Nine of the 12 patients (75%) with surgical excision had at least moderate facial weakness (House-Brackmann grade greater than III) at presentation. Of the 3 patients brought to surgery with normal facial function, 2 cases (Nos. 8 and 12) were misdiagnosed as acoustic neuroma, as it was not known that the tumor arose from the facial nerve until after tumor dissection had begun and electrical stimu-

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TABLE 1. PATIENT AND TUMOR CHARACTERISTICS IN CASES WITH SURGICAL EXCISION (N = 12)

Pt. No.	*Age (y)*	*Sex*	*Major Symptoms*	*Facial Nerve Segments Involved*	*Preop House-Brackmann[5] Grade*
1	32	M	Facial paralysis, conductive HL	CPA to MS	VI
2	47	M	Recurrent facial palsy	IAC to TS	IV
3	42	F	Otalgia, tinnitus	IAC to ET	I
4	13	M	Conductive HL, facial palsy	CPA to ET	IV
5	29	F	Facial palsy	TS to ET	V
6	3	M	Facial paralysis	IAC to GG	VI
7	32	M	Facial paralysis	CPA to MS	VI
8	54	F	SNHL, vertigo	CPA	I
9	42	F	Facial palsy	CPA to TS	IV
10	41	F	SNHL, facial paralysis	CPA, IAC	VI
11	64	M	Facial paralysis	TS to ET	VI
12	54	F	SNHL, imbalance	CPA, IAC	I

HL — hearing loss, CPA — cerebellopontine angle, MS — mastoid segment, IAC — internal auditory canal, TS — tympanic segment, ET — distal extratemporal facial nerve, GG — geniculate ganglion, SNHL — sensorineural hearing loss.

TABLE 2. PATIENT AND TUMOR CHARACTERISTICS IN CASES MANAGED CONSERVATIVELY (N = 10)

Pt. No.	*Age (y)*	*Sex*	*Major Symptoms*	*Facial Nerve Segments Involved*	*Preop House-Brackmann[5] Grade*
13	58	F	SNHL	CPA, IAC	I
14	48	M	SNHL	CPA, IAC	I
15	63	F	SNHL, tinnitus	CPA, IAC	I
16	59	F	SNHL, tinnitus	CPA, IAC	I
17	52	M	Recurrent vertigo	IAC	I
18	20	M	Vertigo, tinnitus	CPA, IAC	I
19	45	M	Conductive HL	GG	I
20	39	F	SNHL, imbalance	CPA, IAC	I
21	45	M	SNHL, tinnitus	CPA, IAC	I
22	54	M	SNHL, tinnitus	IAC to GG	I

lation of the nerve was lost. One patient (case 3) with a very bulky tumor underwent surgical excision in accordance with her wishes.

The 10 patients managed conservatively all presented with normal facial function. One had a conductive hearing loss (case 19), while the others all had symptoms suggestive of more central auditory lesions.

Tumors involving facial nerve segments peripheral to the internal auditory canal, for which expansive growth was hindered by the bony fallopian canal, were much more likely to present with facial dysfunction. Only 1 (case 10) of 11 patients (9%) with central lesions had facial weakness.

Treatment and follow-up facial function are summarized for all 22 cases in Tables 3[5] and 4.[5] Complete tumor excision was carried out by the translabyrinthine approach, except in each of 2 cases (cases 5 and 11) in which the schwannoma was located completely distal to the geniculate ganglion. The preferred method of facial nerve repair was by interposing a greater auricular nerve or sural nerve cable graft, depending on the necessary length. A facial nerve–hypoglossal nerve anastomosis was performed in each of 2 cases (cases 4 and 8) in which the central stump of the facial nerve was too short to hold a suture.

Eight of the patients with total excision (67%) had partial recovery of facial function. Two have longstanding complete facial paralysis. One of these (case 5) had some return of function, which was lost after a cholesteatoma and severe infection developed on the operated side. The other (case 11) had had complete facial paralysis for almost 5 years before the operation. Case 6 also had had complete paralysis for more than 2 years before the operation, but recovered to grade III facial function.

Nine of the 10 patients (90%) managed conservatively did undergo a surgical procedure. In addition to surgical exploration, 3 patients (cases 19, 20, and 21) had wide decompression of their tumors and surrounding nerve segments. One patient with compression of the brain stem (case 14) had extensive tumor debulking in the cerebellopontine angle (CPA), but a small amount of tumor was left behind to preserve the facial nerve.

The 1 patient (case 22) not operated on originally presented with a unilateral sensorineural hearing loss. While a lesion at the geniculate ganglion was present on the original computed tomographic scan (Fig 1), the diagnosis of facial neuroma was not made until 7 years later, on magnetic resonance imaging (Fig 2).

Only 2 of the patients managed conservatively had

TABLE 3. PROCEDURES AND RESULTS FOR CASES WITH SURGICAL EXCISION (N = 12)

Pt. No.	*Surgical Approach*	*Facial Nerve Repair*	*Follow-up Duration*	*Last House-Brackmann*[5] *Grade*
1	Translabyrinthine	GAN cable graft	11 y	IV
2	Translabyrinthine	Sural nerve cable graft	6 mo	Early
3	Translabyrinthine	Sural nerve cable graft	24 mo	III
4	Translabyrinthine	VII-XII	6 y	IV
5	Transmastoid	Sural nerve cable graft	37 mo	VI
6	Translabyrinthine	GAN cable graft	6 y	III
7	Translabyrinthine	GAN cable graft	12 mo	IV
8	Translabyrinthine	VII-XII	11 mo	IV
9	Translabyrinthine	GAN cable graft	9 mo	IV
10	Translabyrinthine	GAN cable graft	30 mo	III
11	Transmastoid	GAN cable graft	25 mo	VI
12	Translabyrinthine	GAN cable graft	1 mo	Early

GAN — greater auricular nerve, VII-XII — facial nerve–hypoglossal nerve anastomosis.

TABLE 4. PROCEDURES AND RESULTS FOR CASES MANAGED CONSERVATIVELY (N = 10)

Pt. No.	*Surgical Procedure*	*Follow-up Duration*	*Last House-Brackmann*[5] *Grade*	*Tumor Growth on Imaging*
13	Retrosigmoid exploration	39 mo	I	No
14	Retrosigmoid exploration and tumor debulking	44 mo	I	No
15	Retrosigmoid exploration	6 mo*	III	N/A
16	Retrosigmoid exploration and biopsy	20 mo*	II	No
17	Retrosigmoid exploration	10 mo†	I	N/A
18	Retrosigmoid exploration	12 mo†	I	N/A
19	Translabyrinthine decompression	47 mo	I	No
20	Translabyrinthine decompression	42 mo	I	No
21	Translabyrinthine decompression	6 wk†	I	N/A
22	None	10 y§	I	No

N/A — no repeat radiologic examination done as of last follow-up.

*Lost to follow-up.

†Recently treated.

§Regular follow-up and serial imaging not begun until 7 years after initial presentation.

facial weakness. Both (cases 15 and 16) experienced dysfunction immediately after the operation, with partial recovery. Five patients (cases 13, 14, 19, 20, and 22) who were followed from 39 months to 10 years continued to have normal facial function and no tumor growth evident on serial radiologic imaging.

DISCUSSION

The treatment for facial nerve schwannoma is surgical excision. Facial function will inevitably be lost as the nerve becomes compressed by continued growth. Tumors in the CPA eventually will put pressure on the brain stem and cause hydrocephalus.[4,6]

The timing of surgical intervention, however, remains unclear. It appears that facial nerve schwannomas almost always grow slowly.[3,4] The most common presentation is facial palsy,[7-9] but the weakness may be very mild or only intermittent.[7,10] Often, patients present with normal facial function and other neurotologic symptoms.[1,11] It may be many years before the onset of any signs of facial dysfunction, especially if the tumor is located outside the fallopian canal.[3,11]

If the patient has moderate to total facial paralysis, tumor removal and facial nerve reconstruction should proceed without delay, as it is well established that the final functional outcome is dependent on the duration of facial palsy.[3,11] However, as illustrated by case 6, a long-standing facial paralysis does not necessarily portend a poor result.

The patient with minimal or no facial dysfunction presents the greatest dilemma to the surgeon. Some authors advocate early surgical resection.[9] They believe that with continued tumor extension, the surgical approach becomes more difficult, the likelihood of postoperative complications increases, and the recovery of facial function is poorer.[9] The risk of invasion of surrounding structures, such as the otic capsule, increases,[9,11] and eventually the brain stem may become compressed.[4,6]

The major disadvantage of this approach is that the excision of a facial nerve schwannoma almost always requires complete resection of a segment of the facial nerve, resulting in total paralysis for at least 6 months. While O'Donoghue et al[11] reported that in 25% of their patients they were able to preserve at least 50% of the facial nerve intact, this has not been

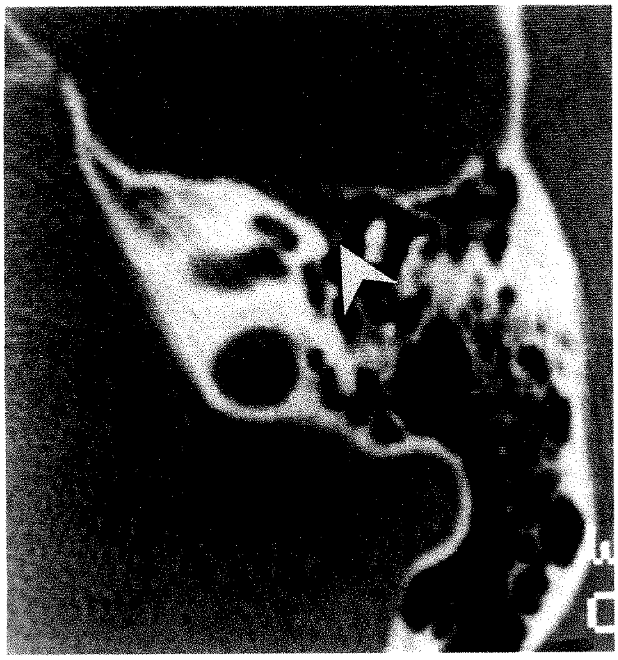

Fig 1. Axial computed tomographic image shows soft tissue density mass (arrowhead) at geniculate ganglion with surrounding bone erosion.

our experience, nor that of others.[3,10,12] Other authors have reported partial nerve resection and the use of onlay grafts,[7,13] but the results do not seem to be better than those of complete resection and cable grafting.[3] Regardless of the timing of surgery or the type of facial nerve repair, the patient can expect no better than an eventual House-Brackmann grade III palsy.

Few patients with normal or near-normal facial function are prepared to accept the outcomes of complete resection. Compounding this reluctance is the fact that most have tumors in the internal auditory canal and CPA and are given a diagnosis of acoustic neuroma before the operation.[1,2] The unexpected finding of a facial nerve schwannoma during the operation is a contraindication to continuing with tumor resection.

An expectant policy has not resulted in any significant complications in our series of 10 patients. All have continued to have normal facial function for up to 10 years after presentation, except for 2 who had partial weakness immediately after the operation. None has shown significant tumor growth on radiologic follow-up.

If surgical excision does become necessary, we prefer the translabyrinthine approach for all schwannomas involving facial nerve segments proximal to the cochleariform process. This approach facilitates complete tumor removal and any grafting techniques used. Therefore, there is little concern regarding otic capsule erosion or more difficult surgical exposure with delay.

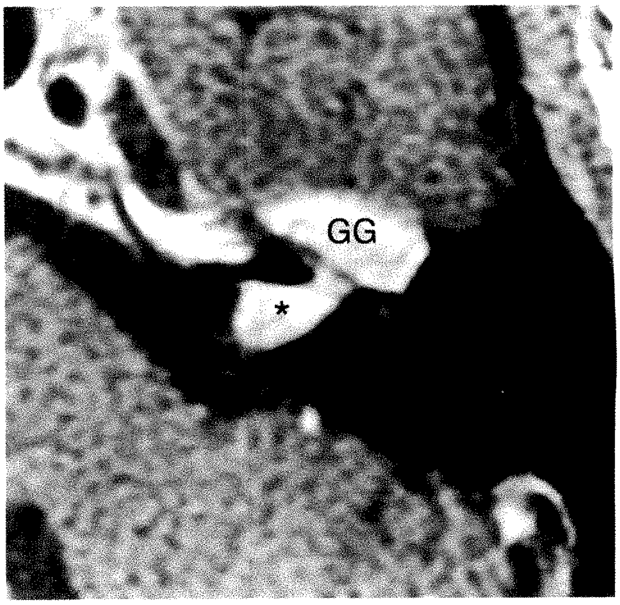

Fig 2. Gadolinium-enhanced T1-weighted axial magnetic resonance image. Enhancing lesion is seen involving left geniculate ganglion (GG) and labyrinthine and intracanalicular segments (asterisk) of facial nerve.

To further delay the necessity of undertaking complete resection, wide tumor decompression has been advocated, allowing tumor expansion outside the natural bony confines.[4] This has been carried out in 3 of our patients through the translabyrinthine approach, once as a planned procedure (case 19) and twice when facial neuromas were unexpectedly found in the internal auditory canal. Additionally, with early impingement on the brain stem, extensive tumor debulking in the CPA may allow the preservation of normal facial function.

The downside to conservative management, especially with central lesions, is that when tumor excision becomes necessary, conditions for nerve grafting may be compromised. As the schwannoma extends toward the brain stem, the proximal facial nerve stump becomes shortened. Eventually, it becomes impossible to suture to it and a facial-hypoglossal nerve anastomosis must be performed, as we experienced in 2 cases.

CONCLUSION

For patients with facial nerve schwannoma and mild or no facial dysfunction, the preferred treatment is often observation and serial radiologic imaging. The short-term results, with respect to facial function, are clearly better, and nothing may need to be done for many years. Tumor debulking and wide decompression are techniques that may allow further delay of definitive resection.

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JACOBSON'S NERVE SCHWANNOMA: A RARE MIDDLE EAR MASS

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The case of a 55-year-old woman with a middle ear mass is presented. The preoperative diagnostic workup, including an audiogram and imaging studies, and the histopathologic findings of the tumor are reviewed. The tumor, a schwannoma, arose from Jacobson's nerve in the middle ear. The surgical anatomy of Jacobson's nerve and the surgical approach to this tumor and to other tumors of the middle ear space are discussed. Tumors of the tympanic cavity are rare, with the exception of cholesteatoma; otherwise, the most common among them are paraganglioma and facial nerve neuroma. This report represents the first documented case of a schannoma arising from Jacobson's nerve in the tympanic cavity.

KEY WORDS — Jacobson's nerve, middle ear mass, schwannoma.

INTRODUCTION

Schwannomas of the temporal bone most commonly arise from the vestibular nerve in the cerebellopontine angle or the internal auditory canal. These neoplasms can also arise from the cochlear nerve, facial nerve, and, theoretically, any location in which peripheral nerves run. Schwannomas are benign tumors of Schwann cells, cells responsible for the myelin sheath that surrounds and protects axons. Schwann cells are found around peripheral nerves; oligodendroglia surround central nervous system axons. Neoplasms of Schwann cells are benign, but cause symptoms as they grow and put pressure on surrounding structures. For example, hearing becomes impaired in patients with vestibular schwannomas as the tumor compresses the cochlear nerve, usually in the internal auditory canal. Other than vestibular schwannomas, schwannomas of the temporal bone are quite rare; facial and cochlear nerve schwannomas are the only other tumors that have been reported with any frequency.

Tumors of the middle ear are also relatively uncommon. A number of different neoplasms, including schwannomas, can originate from the various types of cells in the tympanic cavity. A mass behind an intact tympanic membrane could be epithelial or mesenchymal in origin, benign or malignant. Tumors include cholesteatoma, neuroma or schwannoma, paraganglioma, carcinoma (squamous cell), osteoma or osteosarcoma, adenoma or adenocarcinoma, lymphoma, chondroma or chondrosarcoma, and rhabdomyosarcoma.[1] There have even been documented cases of acoustic neuromas presenting as middle ear masses.[2,3] Most neurogenic tumors arising in the middle ear are facial nerve neuromas.[4-10] We present the first documented case of a schwannoma of the tympanic nerve (Jacobson's nerve) found in the middle ear.

CASE REPORT

A 55-year-old woman presented with left-sided hearing loss and intermittent otalgia. She denied a history of dizziness, imbalance, or otorrhea. Physical examination revealed a mass within the middle ear space with an intact but bulging tympanic membrane. An audiogram showed normal hearing on the right and a large mixed hearing loss on the left. The 4-frequency pure tone average was 80 dB for air conduction and 39 dB for bone conduction, for an air-bone gap of 41 dB (Fig 1). The speech reception threshold was 10 dB in the right ear and 70 dB in the left ear; speech discrimination was not tested at the time. The facial nerve function was normal, as were the remainder of the neurotologic findings.

The radiographic evaluation included computed tomography and magnetic resonance imaging. The computed tomographic examination demonstrated a mass confined to the middle ear space, with focal erosion of the promontory (Fig 2). Magnetic resonance imaging showed solid contrast enhancement of this mass (Fig 3).

The patient was taken to the operating room for middle ear exploration with intact canal wall tympanomastoidectomy and a facial recess approach. The findings at the time of surgery included facial nerve dehiscence along the entire tympanic segment, carot-

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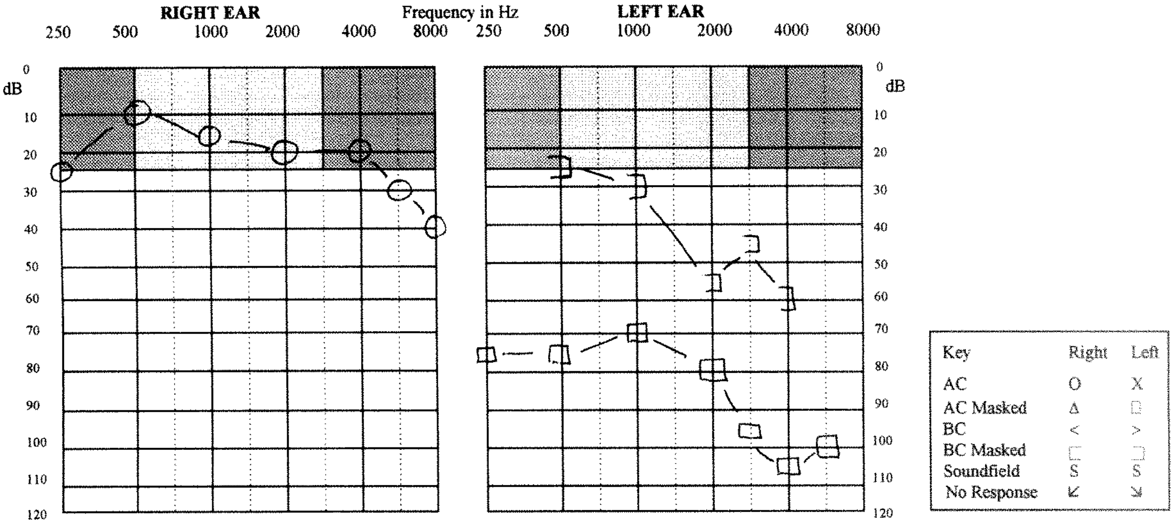

Fig 1. Audiogram of presented patient.

id artery dehiscence and exposure, and promontory erosion with exposure of the cochlea; however, the endosteum appeared intact. The scala tympani and scala vestibuli were visible, but no perilymph fistula was seen through the erosion of the promontory. The long process of the incus was eroded, with ossicular discontinuity. The stapes superstructure was eroded, with only the stapes footplate remaining.

The tumor was first carefully peeled down from the epitympanum and separated from the dehiscent tympanic segment of the facial nerve. It should be noted that the tumor was not of facial nerve origin. Inferiorly, the dissection continued to the oval window area. The stapes superstructure was eroded, and the tumor was easily dissected off the footplate. An encapsulated mass was then delivered through the middle ear by dissection down into the hypotympanum and anteriorly lifting the mass off the promontory. A complete tumor removal was accomplished. Reconstruction was performed with 0.04-inch silicone rubber placed through the facial recess into the middle ear and bone paté used to reconstruct a small scutal defect; temporalis fascia was used to graft the tympanic membrane.

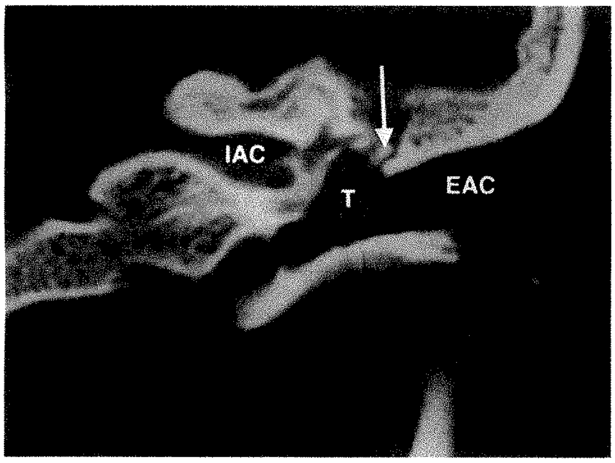

Fig 2. High-resolution coronal computed tomography scan shows mass completely filling middle ear space. Tumor (T) is seen around head of malleus in epitympanum (arrow) and extending toward jugular foramen. IAC — internal auditory canal, EAC — external auditory canal.

Histopathology supported the diagnosis of schwannoma. Grossly, the tumor had a firm, gray cut surface. Microscopically, the tumor had low cellularity. The cells were spindle-shaped and arranged in short fascicles (Fig 4A). Focal, vague nuclear palisading was present (Fig 4B). Thick-walled vessels were scattered throughout the lesion. No mitotic figures were identified. Immunohistochemical staining showed the tumor cells to be positive for S-100 protein, further supporting the diagnosis of schwannoma.

After the operation, the patient did well and had no vestibular symptoms; however, a subsequent audiogram showed that she had a complete sensorineural hearing loss in the ear. The documented sensorineural component on the preoperative audiogram most likely stemmed from erosion of the tumor into the cochlea.

DISCUSSION

As the glossopharyngeal nerve exits the skull base through the jugular foramen, it sends a branch to the middle ear via the tympanic canaliculus. The cell bodies for this branch are located in the inferior ganglion of the ninth cranial nerve. This branch, the tympanic nerve or Jacobson's nerve, ascends along the promontory and provides sensation to the middle ear mucosa and eustachian tube. Referred pain in the ear caused by pharyngeal disease (eg, laryngeal carcinoma) is mediated by this nerve.

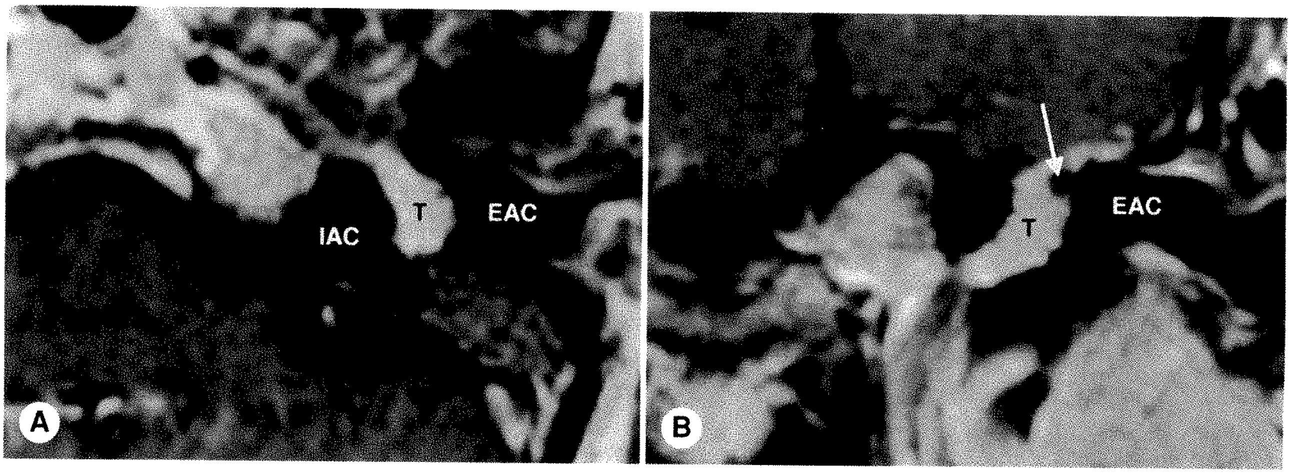

Fig 3. Magnetic resonance imaging shows tumor (T) in middle ear space. **A)** Axial scan after administration of gadolinium pentetate contrast material shows bright enhancement of tumor. **B)** Coronal contrast-enhanced scan. Note low signal intensity of head of malleus in epitympanum (arrow).

Jacobson's nerve is then joined by postganglionic sympathetic branches from the pericarotid sympathetic plexus to become the lesser superficial petrosal nerve. It exits the middle ear through the hiatus of the canal for the lesser superficial petrosal nerve, just lateral to the hiatus of the canal for the greater superficial petrosal nerve. The lesser superficial petrosal nerve sends a twig to the facial nerve at the geniculate ganglion and receives fibers from the greater superficial petrosal nerve. It courses anteromedially to exit the skull base through its own channel or through the foramen ovale. The nerve contains postganglionic sympathetic fibers and preganglionic parasympathetic fibers destined to synapse in the otic ganglion. From the otic ganglion, postganglionic (vasomotor and secretomotor) fibers join the auriculotemporal nerve to the parotid gland.[11,12]

Jacobson's nerve is a peripheral sensory and autonomic nerve whose axons are surrounded by a myelin sheath. The myelin sheath is produced and maintained by Schwann cells. Schwannomas are benign neoplasms that arise from these cells. Although they do not invade bone or tissue as malignancies do, they do cause symptoms and morbidity through local pressure effects. Tumors in the middle ear may cause a conductive hearing loss as they interfere with the ossicular chain, or they may cause a serous otitis media by blocking the eustachian tube. Facial nerve symptoms may also be present with facial nerve neuromas. Pressure effects on the chorda tympani nerve may also cause hypogeusia or other parageusias. Benign tumors may also put pressure on otic capsule bone and result in remodeling and even thinning of the bone, resulting in a fistula with vertigo and sensorineural hearing loss.

The patient in the current study clearly had a schwannoma of Jacobson's nerve, seated directly on the promontory. We believe this tumor arose from Jacobson's nerve because Jacobson's nerve is the only source of Schwann cells in this area, and the tumor did not arise from the facial nerve or the chorda tympani nerve. As the tumor grew, it interfered with the ossicular chain and caused a conductive hearing loss. It also, over time, thinned the otic capsule bone on the promontory, exposing the basal turn of the cochlea and probably producing the sensorineural hearing loss.

The top priority in this case was total tumor removal. This was accomplished through an intact canal wall tympanomastoidectomy with a facial recess approach. As with a glomus tympanicum tumor, the facial recess approach allowed excellent exposure of the middle ear contents all the way anteriorly to the carotid artery and eustachian tube. The canal wall was kept in place to preserve the natural anatomy and to avoid the potential problems and need for follow-up maintenance of an open mastoid cavity; it did not compromise the exposure or removal of the tumor. Additional inferior exposure could have been obtained with an extended facial recess approach, as used for larger glomus tympanicum tumors, but was not necessary in this case.

Cholesteatoma aside, paraganglioma is the most common neoplasm of the middle ear; facial nerve neuroma ranks second.[13] The two should be easily distinguishable on clinical examination and history. Paragangliomas present with pulsatile tinnitus, hearing loss, and a bright red mass behind the tympanic membrane. Facial nerve neuromas also present with hearing loss and tinnitus.[4] Interestingly, facial nerve neuromas do not necessarily present with facial palsy[4,8]; in one study, only 46% of 48 patients with facial nerve neuromas had facial weakness.[4] In this same study, only 29% were found to have a mass

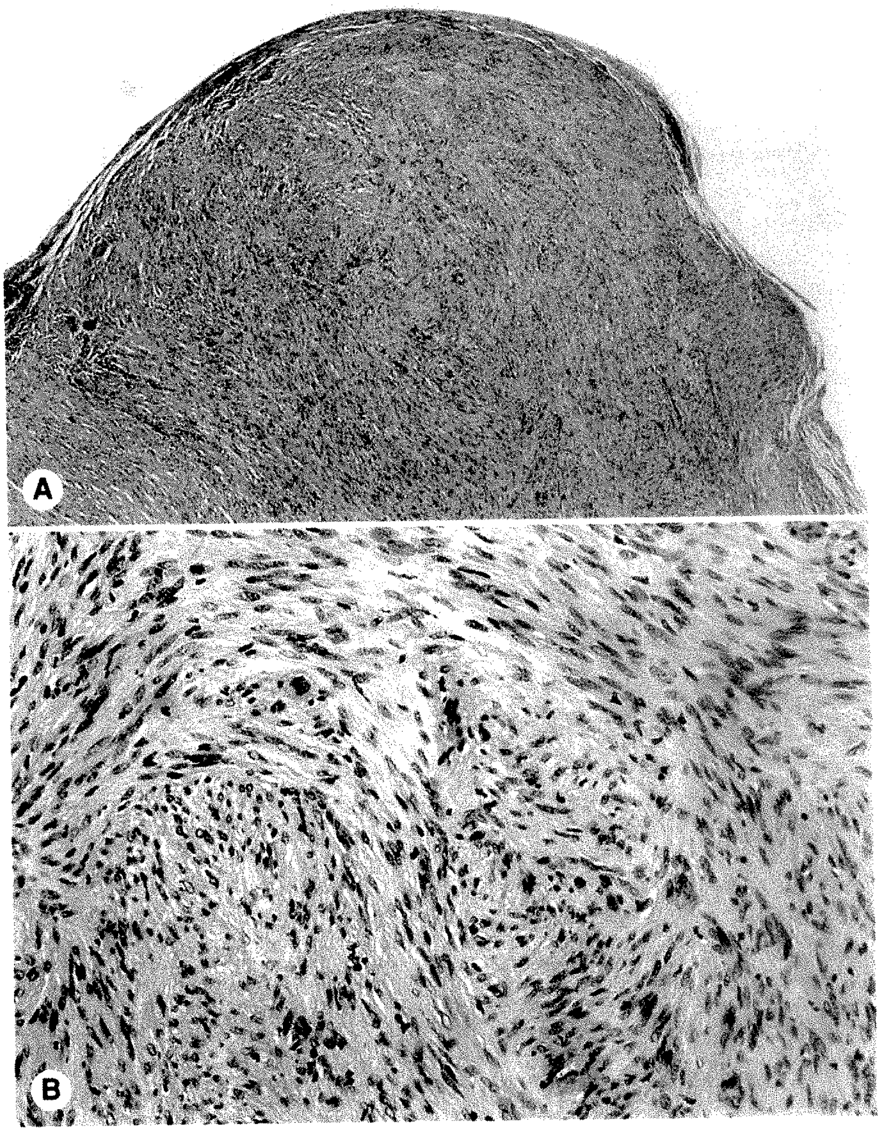

Fig 4. Histopathology of tumor. **A)** Low-power view shows well-circumscribed nodular lesion composed of spindle-shaped cells (original ×40). **B)** Higher-power view shows tumor cells with mild hyperchromasia and nuclear pleomorphism (original ×400). Vague nuclear palisading is also noted.

behind the tympanic membrane. One important point has been documented clearly: if a facial nerve neuroma is suspected, the lesion should not be submitted to biopsy, as biopsy can result in complete facial paralysis.[4,14,15] The diagnosis and management of the mass behind an intact tympanic membrane includes a history, a physical examination with a tuning fork examination, an audiogram, and radiographic imaging prior to definitive treatment.

SURGICAL APPROACHES FOR GLOMUS TUMORS

Classification	*Surgical Approach*
Tympanic	Transcanal
Tympanomastoid	Mastoid–extended facial recess
Jugular bulb	Mastoid-neck (± limited facial nerve rerouting)
Carotid artery	Infratemporal fossa
Transdural	Infratemporal fossa and/or intracranial

Adapted from Brackmann and Arriaga.[16]

Radiographic imaging can help narrow the differential diagnosis. It is an important preoperative study to evaluate the extent and size of the tumor and evidence of bony erosion or destruction; imaging can often differentiate benign from malignant neoplasms as well. Characteristics of benign neoplasms include rounded, discrete tumor borders, lack of bony or soft tissue invasion, and well-defined tissue planes. Masses that invade bone or soft tissue and have indistinct tissue planes with irregular borders are more likely to be malignant. Computed tomographic imaging is very good for evaluating bone and bone–soft tissue interface. Magnetic resonance imaging with contrast is better for delineating soft tissue and tumor. (The Jacobson's nerve tumor in the present case was bright on the postgadolinium images.)

Preoperative imaging will also help determine the surgical approach. As with paragangliomas, the surgical approach is determined by the size and extent of the tumor, the tumor origin (tympanicum versus jugulare; tympanic versus facial neuroma), evidence of erosion, and involvement of the major vascular structures in the ear, the carotid artery and jugular bulb.[16] Surgical approaches to middle ear masses include transcanal, tympanomastoidectomy with facial recess, extended facial recess, mastoid-neck (see Table[16]), combined mastoid–middle fossa, and translabyrinthine. The latter two approaches (mastoid–middle fossa and translabyrinthine) are indicated for facial nerve neuromas in ears with good and poor hearing, respectively.[4]

In many cases, the tumor type can only be determined at the time of surgery, and even then, the final diagnosis may be obscure until the final pathological determination is made. Nevertheless, pathologists are usually able to differentiate benign from malignant neoplasms on frozen section, and surgical resection can be planned accordingly. Benign neoplasms should be removed in toto, with care taken to preserve as much of the natural anatomy as possible. Malignant neoplasms need temporal bone resection with margins; function is not as important as complete tumor removal with as minimal serious morbidity (meningitis, facial nerve paralysis) as possible. The benign neoplasm in our patient was completely removed, with normal postoperative facial nerve function. The tumor had eroded otic capsule bone, and hearing was lost, but the patient had a severe to profound mixed hearing loss before the operation, so she did not experience significant additional loss after surgery.

Tumors of the middle ear can be quite varied. Paraganglioma is the most common, followed by facial nerve neuroma. This is the first documented case of a neuroma of the tympanic, or Jacobson's, nerve. The goals of management are similar to those for any benign neoplasm: gross total removal, minimal morbidity, and preservation of key adjacent structures. Diagnosis and management of any middle ear mass is reliant on a good history, physical examination, audiogram, and preoperative imaging. Biopsy is not necessary and can be injurious; if a facial nerve neuroma is suspected, we complement our preoperative evaluation with electroneuronography. The surgical approach is then carefully planned on the basis of the presumptive diagnosis, audiogram, and imaging studies.

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SERUM LEVEL OF MACROPHAGE MIGRATION INHIBITORY FACTOR AS A USEFUL PARAMETER OF CLINICAL COURSE IN PATIENTS WITH WEGENER'S GRANULOMATOSIS AND RELAPSING POLYCHONDRITIS

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Novel biological activities of macrophage migration inhibitory factor (MIF) have been rediscovered. In addition, elevation of the serum MIF level has been reported in different types of disorders, including various inflammatory and autoimmune diseases. In the present study, serum MIF levels were analyzed in patients with Wegener's granulomatosis (WG) and relapsing polychondritis. It was shown that the serum MIF levels in these patients were significantly higher than those of normal healthy controls. In a WG patient, the MIF level showed a good correlation with clinical symptoms and C-ANCA titers. Thus, serum MIF levels will be a useful laboratory parameter for following the clinical course of WG patients and determining medical treatment. The immunopathologic roles of MIF in these diseases are discussed.

KEY WORDS — C-ANCA, macrophage migration inhibitory factor, relapsing polychondritis, vasculitis, Wegener's granulomatosis.

INTRODUCTION

Macrophage migration inhibitory factor (MIF) is the first described lymphokine that is secreted from activated T lymphocytes in vitro.[1] It inhibits random migration of macrophages and detains them at the inflammatory region. It has been thought that MIF plays an important role in inflammatory and immunologic events. Since the complementary DNA (cDNA) sequence of MIF was determined, a wide distribution and variety of biological functions of MIF have been reported.[2,3] In addition, it has been found that serum MIF levels are elevated in different types of disorders in human and murine disease models.[3-5] It is now generally accepted that MIF plays a critical role in various biological responses, including inflammation and immune responses.

Wegener's granulomatosis (WG) is characterized by granulomatous vasculitis of the upper respiratory tract and lung, glomerulonephritis, and disseminated vasculitis of both arteries and veins. However, the cause is still unclear.[6] Apart from this diagnostic triad, Carrington and Liebow[7] established a concept of "limited forms" of WG in patients without evidence of glomerulonephritis. Since van der Woude et al[8] demonstrated the usefulness of the cytoplasmic pattern of anti-neutrophil cytoplasmic antibody (C-ANCA) staining in determining the patient's condition, the clinical diagnosis of WG has undergone a dramatic improvement, which has enabled the early detection and immediate treatment of the disease with immunosuppressants.

On the other hand, relapsing polychondritis (RP) is a rare disease of unknown cause with a poor prognosis. The first symptom of RP is auricular pain, redness, or swelling. In the course of the disease's progression, the cartilage of the whole body is hampered systemically.[9] It has been proposed that immune responses to type II collagen are involved in the pathogenesis of RP.[10,11] However, it remains to be found whether the titer of autoantibody to type II collagen is elevated in all RP patients. Thus far, there is no specific examination for direct diagnosis of RP, and the delay of diagnosis appears to be related to the poor prognosis.

In the present study, serum concentrations of MIF were quantitated in WG and RP patients. We found elevated serum MIF levels in these patients. It will be discussed whether MIF can be used to determine the pathophysiological condition of WG.

PATIENTS AND METHODS

Twelve patients with untreated WG who visited

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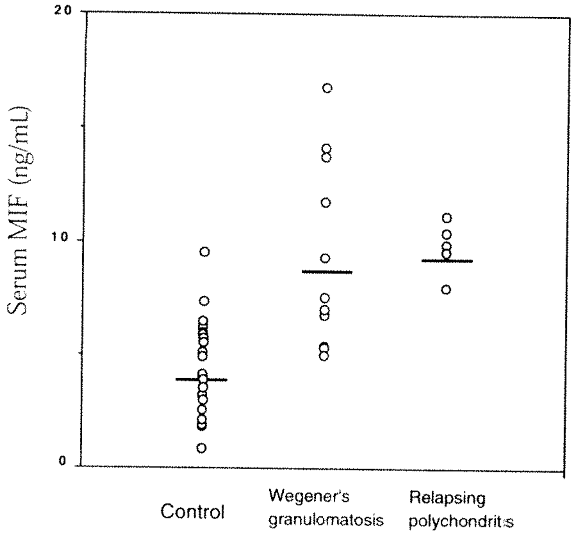

Fig 1. Serum macrophage migration inhibitory factor (MIF) levels of normal healthy controls and patients with Wegener's granulomatosis (WG) and relapsing polychondritis (RP). For WG and RP versus controls, p < .01. Open circles — serum MIF levels of individuals; horizontal lines — mean concentrations of MIF in each group.

Hokkaido University Hospital from 1991 to 1999 were examined (8 men and 4 women, 21 to 72 years old). The criteria for WG diagnosis were 1) clinical symptoms suspect for WG (including refractory rhinitis or sinusitis accompanied by granulosis, saddle nose, perforation of the nasal septum, granulosis, ulcer or stenosis of the subglottic region, otitis media with granulosis, lung shadow accompanied by cavitation, ulcer of the cornea, scleritis, and any other granulosis or vasculitis of unknown origin) with more than 10 U of C-ANCA, or 2) clinical symptoms suspect for WG in those who showed histopathologic evidence of WG.

Five patients with untreated RP (1 man and 4 women, 56 to 69 years old) who visited Hokkaido University Hospital from 1991 to 1999 were examined. The RP diagnostic criteria of Damiani and Levine[12] were used. A diagnosis of RP was made in patients who had 1) 3 or more of the following signs, 2) 1 or more signs with histologic confirmation, or 3) involvement of 2 or more separate anatomic locations with response to steroids and/or dapsone: bilateral relapsing auricular chondritis, nonerosive inflammatory polyarthritis, chondritis of the nasal cartilage, ocular inflammation, chondritis of the respiratory tract, and cochlear and/or vestibular damage. Informed consent for this study was obtained from all the patients examined.

The serum MIF level was determined by enzyme-linked immunosorbent assay as described elsewhere.[4] Briefly, anti-human MIF antibody was added to each well of a 96-well microtiter plate and left for 1 hour at room temperature. All wells were then filled with phosphate-buffered saline solution containing bovine serum albumin (1%) and left for 1 hour at room temperature for blocking. Samples were then added in triplicate to the individual wells and incubated for 1 hour at room temperature. After the plate was washed, 50 μL of biotin-conjugated polyclonal anti-human MIF antibody (4 μg/mL IgG) was added to each well. The anti-human MIF antibody was generated by immunizing New Zealand White rabbits.[4,13] After incubation for 1 hour at room temperature, streptavidin–horseradish peroxidase–conjugated goat anti-rabbit IgG antibody was added to each well and left for 1 hour at room temperature. Thereafter, 50 μL of substrate containing *o*-phenylenediamine and hydrogen peroxide in citrate-phosphate buffer (pH 5.0) was added to each well. After incubation for 20 minutes at room temperature, the reaction was stopped with 4N sulfuric acid. The absorbance at 492 nm was measured.

For determination of serum C-ANCA, a C-ANCA titration kit (PR3-ANC, Nipro, Osaka, Japan) was used. Titration was performed according to the manufacturer's protocol. In brief, sera were diluted 75× by the dilution liquid. Then, 200 μL of these materials or standard solution (10, 100, and 1,000 U) were added to each antigen (purified proteinase 3, which is contained in the cytoplasmic α-granule of human neutrophils)–bound microplate, and incubated for 1 hour at 25°C. The wells were washed 3 times, and 200 μL of alkaline phosphatase-labeled anti-human IgG was added to each well and incubated for 1 hour at 25°C. Then the wells were washed 3 times, and *p*-nitrophenylphosphate was added. These wells were incubated for 1 hour at room temperature, and the absorbance at 405 nm was measured. A standard curve was calculated with standard solutions, by which the patient's C-ANCA levels were extrapolated. Measurement was performed in duplicate or triplicate.

The WG patients were considered to be in remission if all the clinical and radiologic symptoms were absent during the treatment. Disease was considered to be in the exacerbation stage when clinical symptoms increased with or without involvement of additional organ systems. The level of disease activity was based on the clinical activity only, regardless of C-reactive protein, erythrocyte sedimentation rate, or C-ANCA titers.

RESULTS

The mean serum MIF level of the 22 normal healthy controls (age range, 21 to 58 years) was 4.7 ± 0.4 ng/mL (mean ± SE; Fig 1). In contrast, the mean

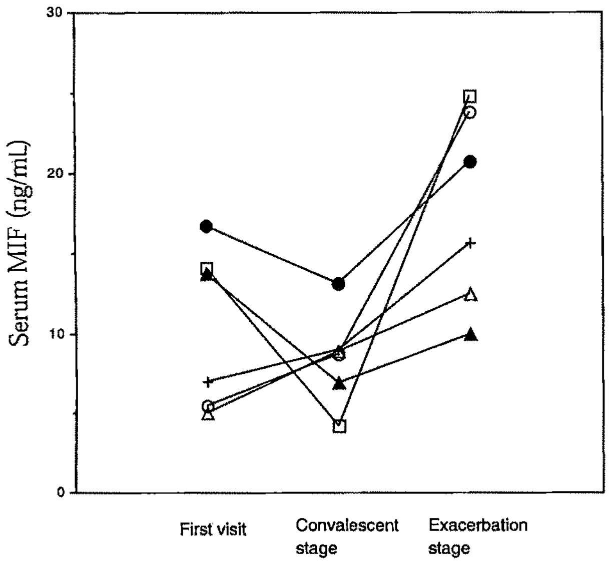

Fig 2. Serial observations of serum MIF levels of WG patients in different clinical stages. Serum MIF levels of 6 WG patients who had disease exacerbations during treatment were sequentially analyzed at their first visit, in convalescent stage, and in exacerbation stage. Closed symbols — patients with diabetes mellitus who were treated with cyclophosphamide or nonsteroidal anti-inflammatory drugs; open symbols — patients treated with glucocorticoids.

MIF level of the 12 WG patients was 9.0 ± 1.2 ng/mL, and that of the 5 RP patients was 9.8 ± 0.5 ng/mL. As compared with the control group, the mean serum MIF levels of the WG and RP patients were significantly elevated (Mann-Whitney U test, $p < .01$).

We then examined whether the serum MIF level was correlated with clinical symptoms. Clinical symptoms of WG and RP patients wax and wane in the natural course of the diseases. Among 12 WG patients, 6 patients had exacerbations during treatment. The mean serum MIF levels of these 6 patients were 10.3 ± 2.1 ng/mL, 8.5 ± 1.2 ng/mL, and 17.9 ± 2.5 ng/mL, at the first visit, after treatment (convalescent stage), and in the exacerbation stage, respectively (Fig 2). The difference between the convalescent stage and the exacerbation stage was statistically significant (Wilcoxon signed-rank test, $p < .05$). Since 2 of the 6 patients (Fig 2) had diabetes mellitus, these patients were treated with cyclophosphamide or nonsteroidal anti-inflammatory drugs (NSAIDs). The other 4 patients were treated with glucocorticoids. The serum MIF levels of these 2 patients were decreased when the symptoms were improved, but after exacerbation, the MIF levels were again elevated. Three of the 4 patients treated with glucocorticoids showed relatively high serum MIF levels even in the remission stage after treatment. In addition, the MIF levels were further elevated in the exacerbation stage. The other patient who received glucocorticoid exhibited a low MIF level during the convalescent stage, but the MIF level was prominently elevated after exacerbation. This patient is described in detail below.

To examine sequentially the relationship among serum MIF concentrations, clinical symptoms, and C-ANCA levels, a case of WG was retrospectively studied. The patient was a 37-year-old woman. She had fever and general fatigue lasting 3 weeks and visited a department of internal medicine. The chest X-ray film showed an abnormal shadow. She received antibiotics, which caused no improvement in

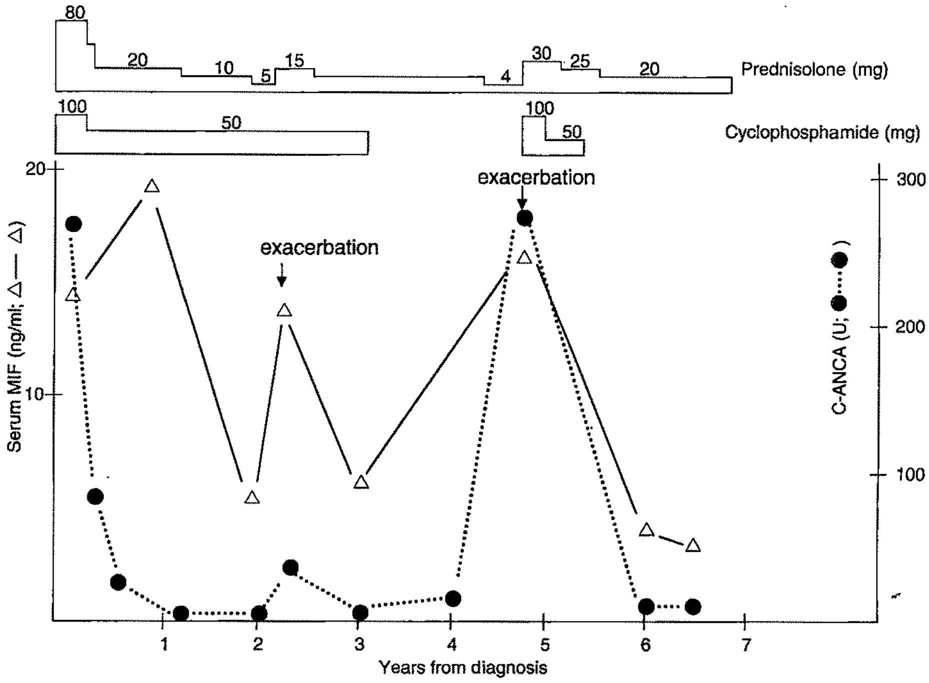

Fig 3. Case summary of WG patient. Triangles — titers of serum MIF level (left axis); circles — titers of C-ANCA level (right axis). Doses of prednisolone and cyclophosphamide are indicated at top.

the clinical manifestations. Then, open lung biopsy was performed and the diagnosis of WG was confirmed. When this patient was first examined in the otolaryngology–head and neck surgery department of Hokkaido University Hospital, her C-ANCA titer was 256 U, and immunosuppressive therapy using 80 mg of prednisolone acetate and 100 mg of cyclophosphamide was initiated (Fig 3). The abnormal shadow in the lung disappeared after this treatment, and the drug doses were gradually reduced. However, more than 2 years later, general fatigue and arthritis emerged, and the C-ANCA titer was concomitantly elevated. Five years after the diagnosis, a second exacerbation began with pneumonia-like symptoms, and an elevated C-ANCA titer was found (Fig 3).

The serum MIF level of the patient was 14.1 ng/mL at the first diagnosis, and was elevated to 19.2 ng/mL 1 year after initiation of treatment with steroids. The MIF level was then decreased to 6.4 ng/mL 2 years after diagnosis, and the symptoms had disappeared. However, the MIF level was again elevated in the first (14.0 ng/mL) and the second (16.6 ng/mL) exacerbation stages (just before the steroid increment). These findings demonstrate that the serum MIF level changes in parallel with the titer of C-ANCA (Fig 3) and with the clinical manifestations, although the first peak of the MIF level was observed when the patient was in the convalescent stage.

DISCUSSION

In WG patients, the mean serum MIF level was significantly higher than that in normal healthy controls. When these patients were treated with NSAIDs, the MIF level was slightly decreased. In contrast, most WG patients who received steroids showed no reduction in MIF. At any rate, the serum MIF level was markedly increased in patients during the exacerbation stage of the disease. Immunosuppressants such as glucocorticoids and cyclophosphamide are commonly used to treat WG. It has been reported that MIF acts as a counter-regulator of glucocorticoids.[14] We found elevated serum MIF levels in patients with Vogt-Koyanagi-Harada syndrome immediately after steroid treatment (unpublished observations). Indeed, in the present study, the MIF levels appeared to be upregulated in WG patients after the initial treatment with glucocorticoids. A similar observation was obtained in a WG patient whose clinical course was followed for 7 years (Fig 3). The MIF level was elevated to its peak after the initial treatment with prednisolone and then decreased during reduction of the prednisolone dose (convalescent stage). Thereafter, however, the MIF level was elevated during 2 exacerbation stages irrespective of the prednisolone dose. After the second and third prednisolone increments, the MIF level decreased. The relationship between steroid treatment and the MIF levels seen in this particular patient is unclear at present.

It has been reported that C-ANCA fluctuates irrespective of disease activity.[15] In the present study, however, the C-ANCA titer in a particular patient showed peaks in accordance with the elevation of serum MIF (Fig 3). Furthermore, in the other WG patients of this report, increased C-ANCA titers were consistently observed in the exacerbation stages (data not shown). Thus, it seems to us that the serum MIF level, the C-ANCA titer, and the clinical symptoms show a good correlation. Currently, C-ANCA is widely used as one of the parameters for following the condition of WG patients, and the present findings indicate that the serum MIF level is also useful.

Recently, the ANCA cytokine sequence theory was proposed to explain the immunopathologic role of ANCA in WG.[16] In this postulate, ANCA itself not only induces vasculitis, but also enhances the expression of adhesion molecules on the vascular endothelial cells. A minor proportion of proteinase 3 (the target of C-ANCA) appears to be expressed in the plasma membrane of neutrophils and monocytes, as well as in azurophilic granules. The binding of ANCA to neutrophils results in degranulation of proteinase 3, myeloperoxidase, and reactive oxygen species and eventual activation of these neutrophils. In this way, adhesion of activated neutrophils and monocytes to endothelial cells is induced, which leads to vasculitis and necrosis.

Also, ANCA lyses vascular endothelial cells when incubated with proteinase 3 or myeloperoxidase and neutrophils. Reverse transcription–polymerase chain reaction/Southern blot analysis revealed that MIF was expressed in human vascular endothelial cells.[13] Thus, MIF appears to be released from damaged vascular endothelial cells. Then, the released MIF upregulates tumor necrosis factor–α, which may enhance the degranulation of neutrophils together with ANCA.[16] Indeed, an elevated level of serum tumor necrosis factor–α has been reported in WG patients.[17] In addition, macrophages and monocytes that play substantial roles in the pathogenesis of granulomatous inflammatory lesions in WG produce MIF.[18] It seems to us that this vicious circle is involved in the pathogenesis of WG. It should also be noted that the levels of interleukin-8,[19] interleukin-2R,[20] and thrombomodulin[21] are elevated in WG. To elucidate the immunopathologic background of WG requires analysis of the relationship between these components and upregulated MIF, as well as C-ANCA.

In the present study, it was also shown that RP patients exhibited significantly high levels of MIF. Patients with RP sometimes show arthritis and autoantibodies to type II collagen that are similar to those of rheumatoid arthritis. In rheumatoid arthritis, macrophages play major roles[22,23] and produce MIF.[24] Thus, macrophages may also be involved in the pathogenesis of RP. However, autoantibodies to type II collagen are not always detected in RP patients,[10,11] and the relationship between RP and rheumatoid arthritis remains to be elucidated. At any rate, since there is no reliable marker for RP, such as C-ANCA for WG, measurement of the serum MIF level may be of diagnostic assistance. Although hindered by RP's being a rare disease, accumulation of MIF data in RP cases is needed for us to understand the role of MIF in the pathogenesis of RP.

The causes of the two diseases, WG and RP, are still unclear. The present study suggests that MIF plays a certain role in the pathogenesis of these diseases. In a type II collagen–induced arthritis model, mice treated with anti-MIF antibody showed a retardation in the development of arthritis.[5] We have reported that administration of anti-MIF antibodies to rats delayed the onset of experimentally induced uveoretinitis and reduced the histology score.[25] Furthermore, in an experimental model of septic shock in mice, the lethality was significantly reduced when the mice were treated with anti-MIF antibody.[26] These results collectively suggest the possibility that anti-MIF antibodies can be used as a therapeutic agent for these diseases. For examining this possibility, animal models for WG and RP need to be developed and used in future studies of experimental therapeutics.

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BIOLOGY, PREVENTION, AND TREATMENT OF HEAD AND NECK CANCER

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HEAT RADIATION DURING CALORIC VESTIBULAR TEST: THERMOGRAPHIC DEMONSTRATION IN TEMPORAL BONE EXPERIMENTS

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Since Bárány's descriptions in 1906, cold and warm irrigations of the external ear canal have been used for unilaterally testing vestibular excitability. The fluid kinetics within the endolymph have been studied thoroughly, whereas relatively few published articles deal with the mechanisms of heat transfer from the external to the internal ear. Even though heat conduction via the bone seemed to be well established in the textbooks, Feldmann and co-workers found heat radiation to be a very important factor. Using a rather uncommon method, we tried to make this heat radiation more apparent: in temporal bone experiments, temperature distribution was followed by thermography. Temporal bone specimens were prepared in such a way that heat distributions became visible after experimental caloric irrigations of the external ear canal. Temperature changes could well be verified by changes in coloration of the 2-dimensional thermograms. Conclusions were drawn from registrations performed in 2 projections, 90° to each other, allowing cautious statements about 3-dimensional distribution. The velocity of heat transfer at the very onset of the reaction can only be explained by radiation, whereas later, other mechanisms, such as conduction via the bone or convection via the middle ear gas, may have some influence. Our results support Feldmann's findings and underline the significance of radiation in the heat transfer mechanism.

KEY WORDS — calorization, heat transfer, radiation, thermography, vestibular test.

INTRODUCTION

According to Bárány's[1] theory of caloric vestibular stimulation, hot or cold stimuli cause fluid kinetic phenomena mainly within the endolymph of the horizontal semicircular canal. It is not clear, however, how heat is transported from the external ear canal to the labyrinth. Different ideas have been discussed since Bárány's day, most of them focusing on heat conduction through the bone. In 1991, Feldmann et al[2] experimentally demonstrated the significance of heat radiation in this very complex mechanism. Although already, in 1925, Dohlman[3] had mentioned the physical possibility of radiative heat transfer, he apparently regarded it as of minor importance. Some authors have regarded other factors, such as convection, as possible contributors.[4-8] The report of Feldmann et al has been discussed critically, on the grounds that it overemphasized heat radiation, but has not been fundamentally contradicted.[9]

Feldmann et al[2] determined the significance of heat radiation indirectly by removing all other modes of heat transfer from their experiments. They demonstrated that heat transport took place in the absence of all heat transfer mechanisms other than radiation. They minimized heat conduction through the bone by cutting away the bony tympanic walls, leaving only a very slim bony section connecting the external ear to the labyrinth. Simultaneously, they excluded convection, since the model was essentially open and exposed on all sides. Feldmann et al did not claim radiation to be the only factor in heat transfer from the external ear canal to the inner ear. However, they identified radiative heat transfer as a previously ig-

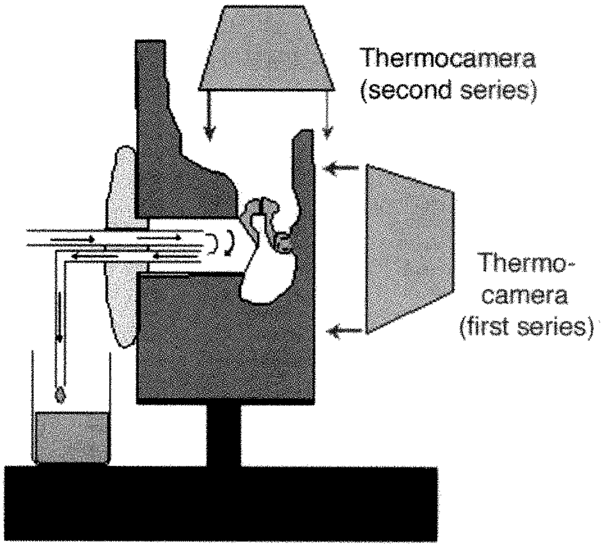

Fig 1. Schematic of experimental arrangement.

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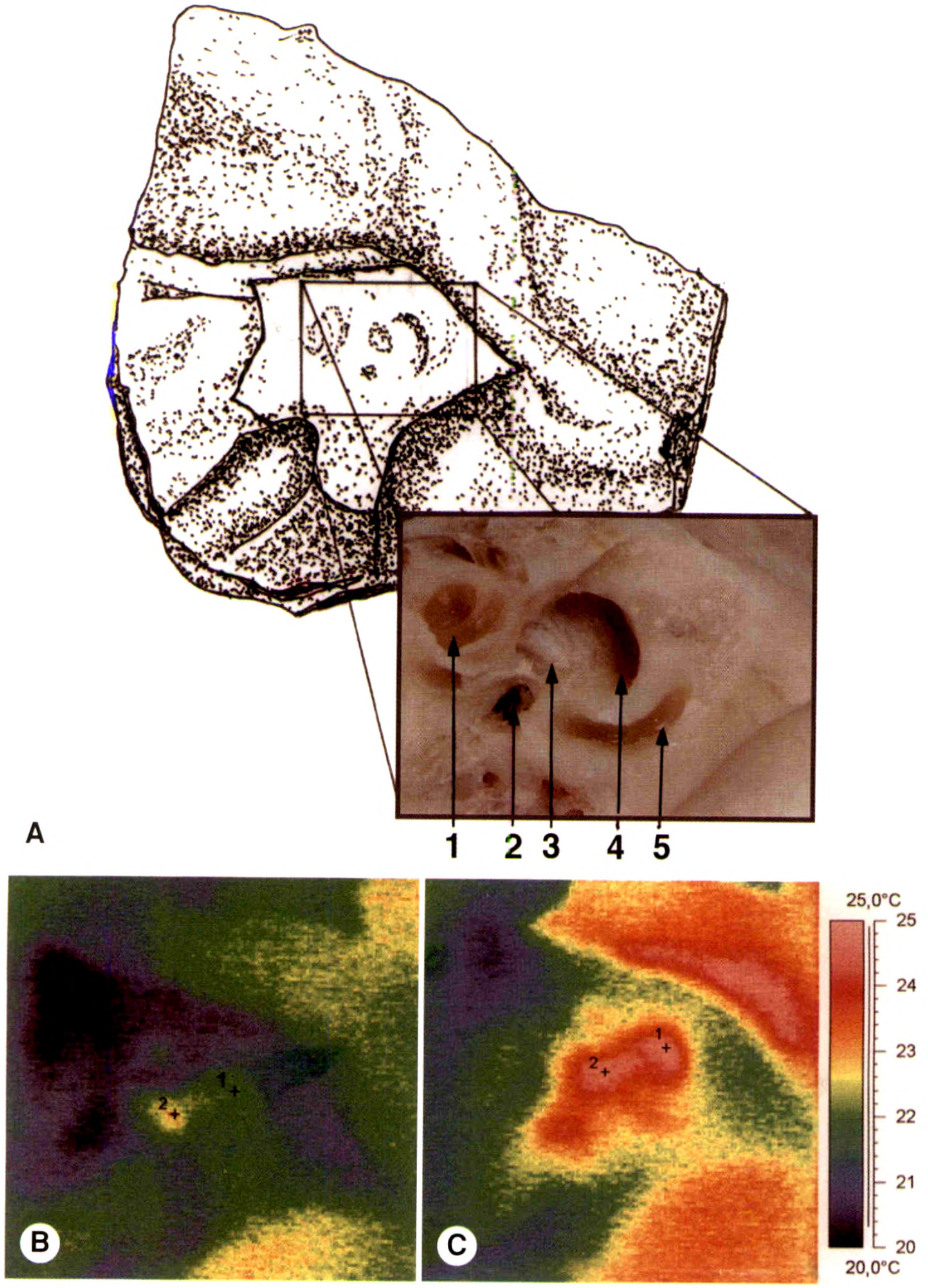

Fig 2. Heat distribution after caloric stimulation in petrous bone model — first series. **A)** Medial aspect of petrous bone with tip of pyramid removed. Vestibulum and semicircular canals are opened. 1 — cochlea; 2 — round window; 3 — oval window; 4 — lateral semicircular canal; 5 — posterior semicircular canal. **B)** Thermogram obtained 12 seconds after start of warm irrigation. Although, as described in text, it was not possible to maintain entire preparation at constant temperature, in this phase, warmer spots (point 1, 23°C) or areas (point 2, 22.2°C) become visible. **C)** Thermogram obtained 2 minutes later shows more intensive coloration in exposed labyrinthine wall (point 1, oval window, 24.8°C; point 2, exposed lateral semicircular canal, 24.8°C).

nored factor in the complex heat transfer process.

Our approach to studying heat radiation was somewhat different from that of Feldmann et al.[2] With a preparation similar to that of Feldmann et al, an infrared thermocamera allowed us to "see" the radiation directly, whereas Feldmann et al had inferred radiation as the only remaining heat transfer mechanism. In a previous study,[10] we were able to optically demonstrate heat distribution in one plane. Adding a second projection (90° to the first) would enable us to be sure we were describing heat transport through the temporal bone correctly. It must be stressed that our experiments are a qualitative demonstration of radiative heat transfer. Quantitative measurements, if possible at all, would require a much more sophisticated experimental arrangement.

MATERIALS AND METHODS

The investigations were done with 2 fresh human temporal bones. The vestibulum and the semicircular canals were opened from the medial side by sawing off the inner portion of the pyramidal part of the petrous bone. Thus, the vestibulum, the cochlea, and the vestibular semicircular canals could be burred open in order to gain exposure of their lateral walls. By removing the tegmen tympani and antri, we exposed the middle ear from above (Figs 1, 2A, and 3A).

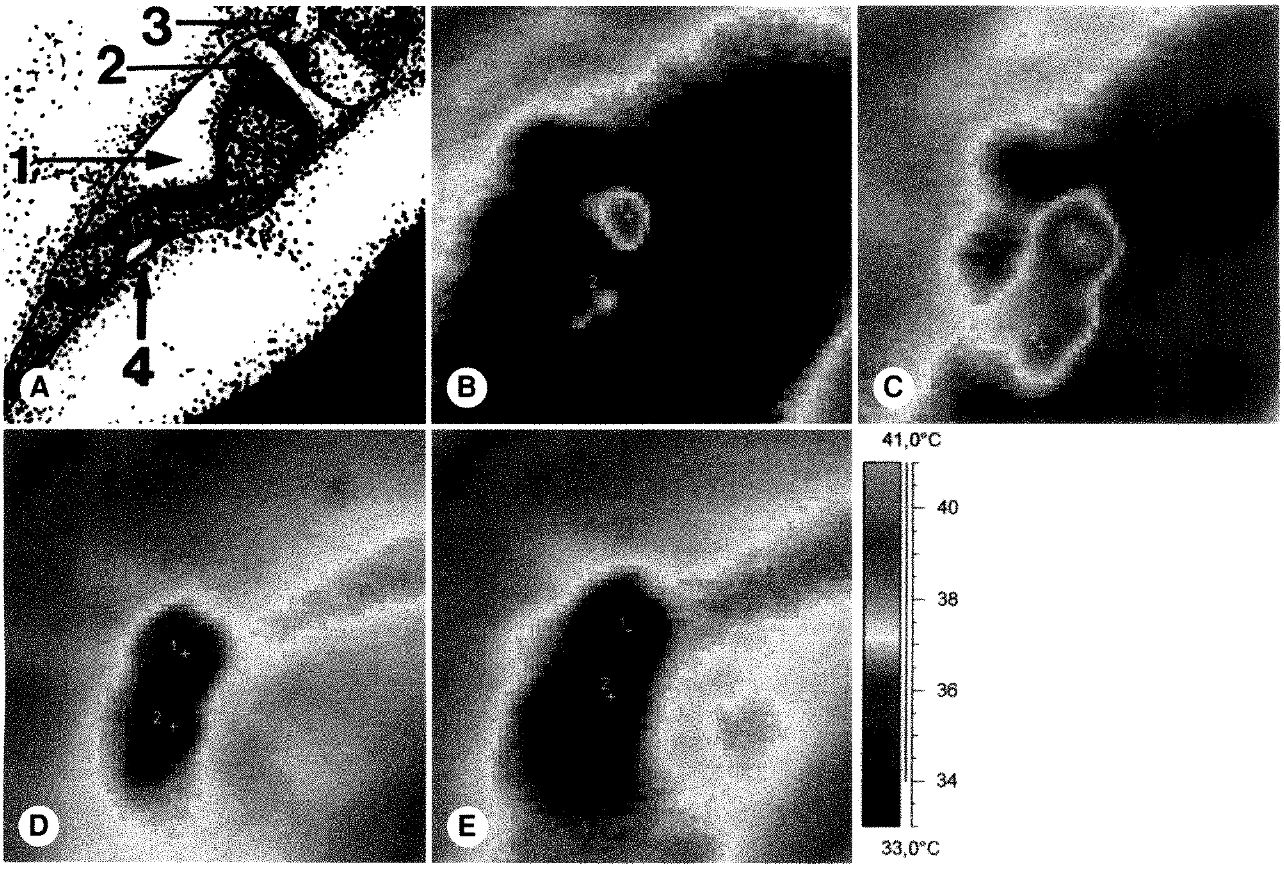

Fig 3. Heat distribution after caloric stimulation in petrous bone model — second series. **A)** View from above into opened middle ear. 1 — tympanic membrane with umbo; 2 — tendon of tensor tympani muscle; 3 — incudostapedial joint; 4 — slice of bone placed on promontory. **B-E)** Thermograms obtained during warm and cold irrigation. As described in text, it was not possible to maintain entire preparation at constant temperature of 37°C. Individual temperature zones can be read from scale at edge of Figures. **B)** Thermogram obtained 3 seconds after start of warm irrigation. Note red area (point 1, 39.0°C) corresponds to tympanic membrane. Other red area (point 2, 37.9°C) is bone chip placed on promontory. **C)** Thermogram obtained 41 seconds later shows more intensive, but also more diffuse, coloration of hottest region (point 1, 41.3°C; point 2, 39.1°C). **D)** Thermogram obtained after cold irrigation. Note simultaneous change in color of tympanic membrane (point 1, 33.5°C) and slice of bone on promontory (point 2, 34.7°C). **E)** Thermogram obtained 24 seconds later (point 1, 33.6°C; point 2, 34.4°C).

By fixing the tympanic membrane with formalin, we delayed degradation of the membrane, thus allowing additional experiments. We performed the first series of experiments (first projection) at room temperature, using 12°C and 32°C water for cold and warm irrigations, respectively. In the second series (second projection), the petrous bones were heated to 37°C. The temperatures of the water used for irrigation were 30°C and 44°C. In each series, 50 mL of water was applied within 15 seconds.

The outer ear canal was sealed and connected to an irrigation system. As already mentioned, the first series was carried out at room temperature, whereas in the second series, a warming chamber was used. Nevertheless, it was not possible to obtain a uniform temperature in all parts of the preparation because of the many external influences such as thermoconvection within the room or the opening of the heating chamber required for using the thermocamera. We considered these systematic faults to be insignificant after comparing different experimental situations. Measurements were performed with the thermovision system AGEMA 470 (FLIR Systems, Frankfurt, Germany), allowing continuous, contactless registration in the form of colored thermograms that were storable and evaluable by computer. In a preliminary test, we estimated the emission coefficient of bone to be 0.96 (accuracy ± 0.01) by comparing bone to materials whose emission coefficients were known. This dimensionless number indicates that the surface examined emits radiation almost like an ideal black body (emission coefficient = 1).

For close-up studies, special modification of the camera system was required. In the first series, thermograms of the lateral wall of the inner ear were taken with the camera "looking" at the temporal bone from its medial side. In the second series, the direction of view was from above, through the open tegmen tympani et antri, allowing a view of the entirety of the middle ear structures. For a less tangential view, a small slice of bone was placed on the promontory and imaged with the camera (Fig 3A).

RESULTS

Although, as has been mentioned, the temperature could not be kept entirely stable, good and comparable thermograms were obtained. In the first series, it could clearly be seen that within a short period of time (12 seconds), there was a marked temperature increase in the opened inner ear, with its maximum at the lateral semicircular canal and the basal turn of the cochlea (Fig 2). In the second series (tympanic membrane and medial wall of the tympanic cavity), it could be seen (Fig 3) that practically at the same moment both structures changed color, indicating changing temperature. After some time, these effects became less distinct and were more difficult to associate with anatomic structures. In both cases, the initial phase of the experiment is of greatest interest: the high heat transfer rate observed at the beginning can only be the result of radiation.

DISCUSSION

The aim of our study was to directly visualize the transport of heat and make the mechanism of heat radiation more readily apparent. Thermography has many advantages over the single temperature probes used previously. Single probes can only measure temperatures at distinct points, have a certain time lag, and can even themselves interfere with heat conduction.

Thermography, in contrast, gives a noncontact, continuous 2-dimensional picture of the area of interest. The camera "sees" the distribution of heat, making the results more plausible to the viewer. While thermography generally is used to show the noncontact surface temperature distribution, temperature information as a function of depth can be obtained on specially prepared samples like ours. The bone specimens were extremely opened, allowing visualization of heat transfer from the external ear canal to the labyrinth in 2 projections angled by 90°. Thus, conclusions about the 3-dimensional distribution, in our opinion, were allowed. On the other hand, our preparation must be examined with caution because of the possibility of artifacts. In our case, the entire tip of the labyrinthine pyramid was removed so that the heat passage from middle to inner ear could be visualized.

In the first series, experiments were done on room-temperature specimens. Nevertheless, it was difficult to keep the surface temperature stable.[10] This was even more difficult to maintain in our second series, in which, for imitating a more "physiological" situation, bone was heated to about body temperature (37°C). Actually, the basic temperature is insignificant for our observations of relative temperature changes.

Of course, these conditions are not physiological. The entire preparation is an abstraction. Despite the many experimental insufficiencies, the results confirm the significance of heat radiation. Radiation is the only explanation for the observed high velocity of heat transfer to the inner ear (first series) and for the fact that in the second series the tympanic membrane and medial wall of the tympanic cavity changed temperature simultaneously. Although it is not intuitively obvious, we have experimentally shown the importance of radiation in the complex heat transfer mechanism from the external ear canal to the inner ear, at least in the very early phase. Later, other factors may gradually become more significant.

A much more sophisticated model would be needed to define the relative contributions of the 3 heat transfer mechanisms: conduction, convection, and radiation.

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BOTULINUM TOXIN TYPE A INDUCES APOPTOSIS IN NASAL GLANDS OF GUINEA PIGS

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Nasal hypersecretion is predominantly caused by overaction of nasal glands, which are mainly under cholinergic control. In this work, we investigated the influence of botulinum toxin A (BTA) on the nasal mucosal tissue of the maxillary sinus turbinates of guinea pigs (n = 10) that were painlessly sacrificed 10 days (short-term group) or 3 months (long-term group) after local treatment with 20 units of BTA (Botox) or 0.2 mL of 0.9% sodium chloride (control). Histologic investigation of the nasal mucosal tissue of the BTA-treated animals (short-term group) showed degeneration of glands and ducts and apoptotic nuclei on TUNEL staining of these structures. The control animals revealed normal glandular tissue and no apoptosis. The animals of the long-term group showed almost normal glandular tissue and only a few apoptotic nuclei. In conclusion, BTA induces temporary apoptosis in the nasal glandular compartment of guinea pigs.

KEY WORDS — apoptosis, botulinum toxin, nasal glands.

INTRODUCTION

Allergic and nonallergic rhinitis are common clinical conditions often treated by otolaryngologists. Besides nasal obstruction and sneezing, one of the cardinal symptoms of rhinitis is the excessive production of nasal fluids, generated mainly by secretion from the nasal glands and by exudation from the mucosal vasculature.

If the stimuli inducing rhinitis are known (ie, allergens like house dust mites and pollen), the hyperreactivity is termed specific. If not, we speak of a nonspecific hyperreactivity. The nonallergic, noninfective forms are often referred to as intrinsic rhinitis.[1]

The pathogenesis of nasal hyperreactivity is as manifold as its entities. In addition to allergic rhinitis (perennial, seasonal, job-related, nutritive), nerve-induced rhinitis, rhinitis caused by medicines (aspirin, antihypertonic preparations), irritative rhinitis (from xylometazoline-containing nasal decongestants, hormone-induced), postinfectious rhinitis, and idiopathic rhinitis without an obvious pathological mechanism may occur. Conditions such as NARES (nonallergic rhinitis with eosinophilia) or nasal mastocytosis need further investigation. Therapeutic options in treating nasal hyperreactivity depend on the cause and pathogenesis of the particular type of rhinitis and the current complaints of the patient, including allergen reduction, treatment with, for example, local decongestants and topical steroids, specific immunotherapy (allergic rhinitis), adaptive deactivation (aspirin-sensitive rhinitis), and rhinosurgical treatment. Since few of the methods described above yield satisfactory results for rhinorrhea, further therapies have to be developed.

Serous secretion in rhinitis is mostly under parasympathetic control and, therefore, should be sensitive to anticholinergic substances. Botulinum toxin is a neurotoxin that inhibits the release of acetylcholine from the presynaptic nerve terminal.[2] It is therapeutically used in otolaryngology for many entities — for example, spasmodic dysphonia, dysphagia, oromandibular dystonia, and facial movement disorders. It has also been used in the autonomic nervous system to treat gustatory sweating[3,4] and sialorrhea,[5] in which a reduction of salivary flow has been demonstrated. Immunohistochemical findings in salivary glands of rats treated with botulinum toxin A (BTA) have shown a decrease in the concentration of acetylcholinesterase,[6,7] indicating that the toxin blocks the cholinergic pathway of parasympathetic innervation.

Botulinum toxin has already been applied to the human nasal cavity by Kim et al.[8] They showed a significant reduction in rhinorrhea in patients with intrinsic rhinitis after local injection of BTA. Shaari et al[9] demonstrated a decrease in rhinorrhea in dogs

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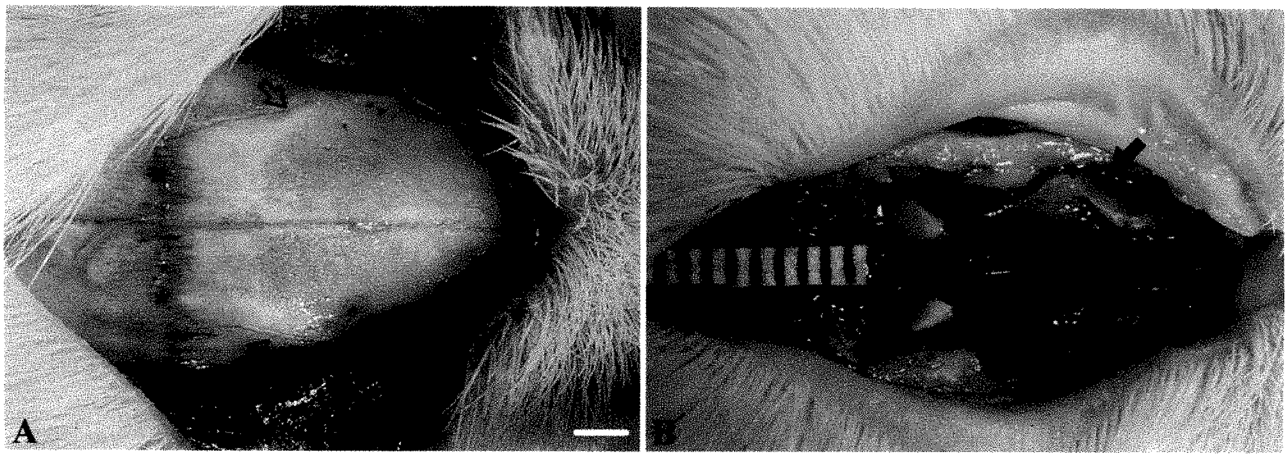

Fig 1. Nasal cavity of guinea pig. Left is rostral, and right is frontal. **A)** Nasalia and adjacent suturae (arrows and asterisks). Scale bar — 0.5 mm. **B)** Left maxillary sinus turbinate (arrows) after removal of nasalia and nasal septum (asterisk). Rule is 1 cm, subdivided into millimeter steps.

after nasal application of BTA.

The cellular changes leading to a reduction in nasal secretion after BTA application have essentially still not been investigated. Knowledge of these changes is, however, of significant relevance in order to design treatment on a rational basis. In this study, we report on the effects of BTA on the nasal turbinate mucosa of guinea pigs.

MATERIALS AND METHODS

Animal Treatment. This study was performed in accordance with paragraph 8, section 1, of the German Animal Protection Law and was approved by the local animal care committee.

Guinea pigs (Dunkin Hartley, n = 10) weighing 500 to 600 g were anesthesized (ketamine hydrochloride 100 mg/kg, xylazine hydrochloride 4 mg/kg), and a small sponge (Merocel, Xomed Surgical Products, Jacksonville, Florida) was introduced into the left nostril and then soaked with 20 units of BTA (Botox, Allergan Inc, Irvine, California; 1 mL of 0.9% sodium chloride in 1 bottle of lyophilized toxin, 0.2 mL = 20 units of BTA). The right nostril was left empty. The sponge was left in place for 1 hour and then removed. Five animals served as controls. The sponge in these guinea pigs was soaked with 0.2 mL of 0.9% sodium chloride and was removed after 1 hour. The animals were painlessly sacrificed 10 days (short-term group; 3 BTA-treated animals and 3 control animals) or 3 months (long-term group; 2 BTA-treated animals and 2 control animals) after treatment. The maxillary sinus turbinates (corresponding to the lower turbinate in humans) on both sides were obtained after removal of the nasalia (corresponding to the os nasale in humans) and the nasal septum (Fig 1).

Preparation of Tissue Sections. After the procedure described above, the samples were fixed in 4% paraformaldehyde in 0.1 mol/L phosphate buffer (pH 7.2) for 24 hours, decalcified in 10% ethylenediaminetetraacetic acid for 1 week at room temperature with daily renewal of the solution, and automatically embedded in paraffin (Shandon Citadel 2000). A microtome (Leica Biocut 2035) was used to cut 5-μm sections, which were mounted on coated glass slides for TUNEL (terminal deoxynucleotidyl-mediated deoxyuridine triphosphate nick end labeling) staining and on uncoated glass slides for histochemistry. The slides were dried overnight at 37°C. To demonstrate possible cellular changes, we used periodic acid–Schiff (PAS) histochemistry and also performed TUNEL staining.

Histochemistry. Periodic acid–Schiff staining for mucous glycoproteins was performed. Sections were deparaffinized in xylol, hydrated in a descending alcohol series, and transferred to distilled water. The sections were immersed in 1% aqueous periodic acid for 10 minutes, rinsed in distilled water, stained with Schiff's reagent (Sigma, St Louis, Missouri) for 15 minutes, rinsed in sodium pyrosulfate, and, finally, washed thoroughly in running water. The sections were counterstained with hematoxylin (Merck, Darmstadt, Germany), dehydrated, and cover-slipped. One section of each maxillary sinus turbinate was chosen for evaluation; all sections were from the same anatomic location in each animal. Zones of affected and unaffected glands were identified by light microscopy. The sizes of the zones of each section of affected and unaffected glands were measured with a semiautomatic image analysis system (MOP AM02, Kontron Electronic, Eching/Munich, Germany). The sizes were then expressed as a percentage of the total size of the zones of all glands measured.

TUNEL Procedure. Apoptosis is characterized by

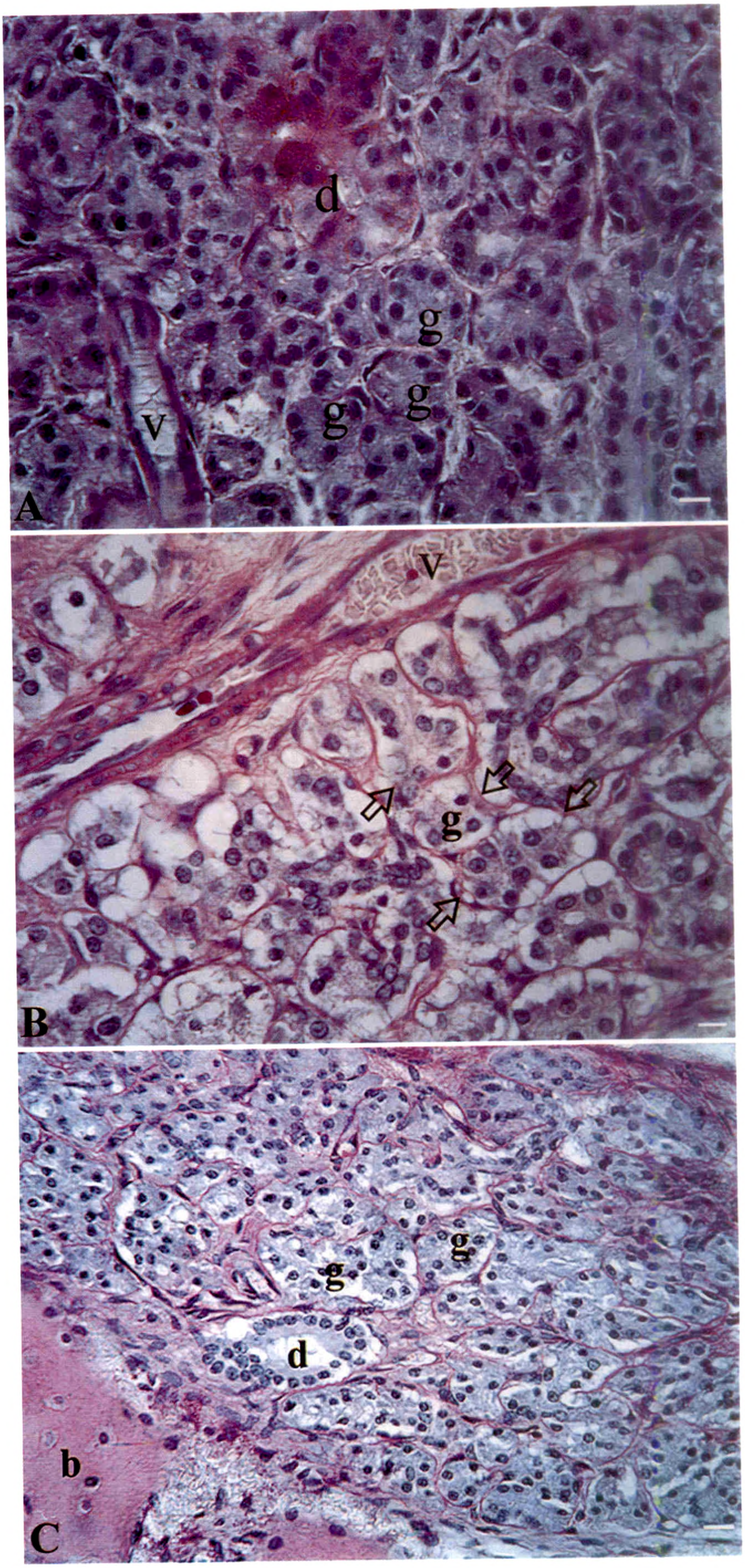

Fig 2. Histochemistry (periodic acid–Schiff stain). **A)** Submucosal glands (g), gland duct (d), and vessel (v) of left maxillary sinus turbinate of short-term control animal represent normally configured glandular tissue. Scale bar — 10 µm. **B)** Submucosal glands (g) and vessel (v) of maxillary sinus turbinate of short-term botulinum toxin A (BTA)–treated animal. Note degeneration of glands (arrows). Scale bar — 10 µm. **C)** Glandular tissue of maxillary sinus turbinate of long-term BTA-treated animal. Almost normally configured submucosal glands (g) and gland ducts (d). b — Bone; scale bar — 20 µm.

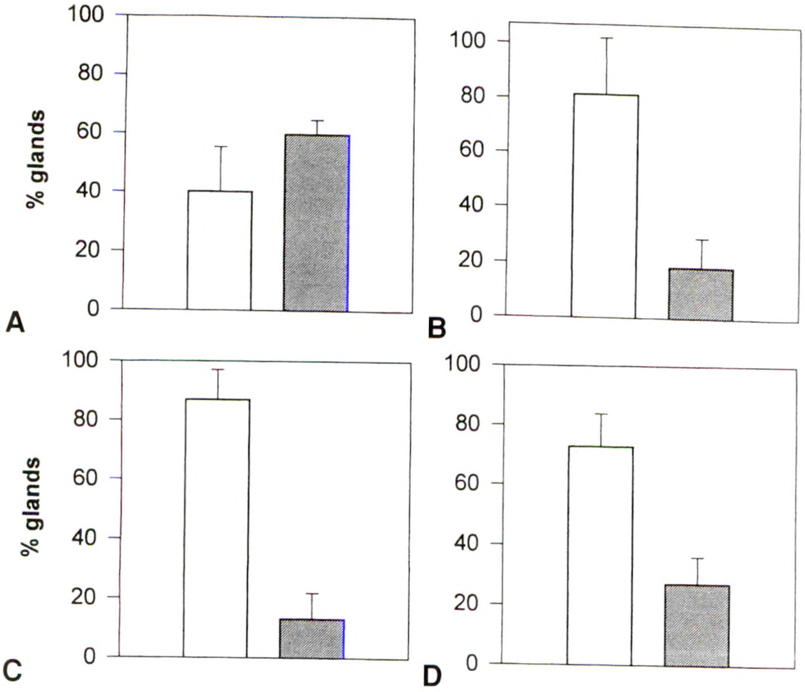

Fig 3. Semiautomatic image analysis with which sizes of areas of normal and degenerated glands were measured and expressed as percentage portion of all glands measured per section. Unfilled columns represent normal glands, filled columns represent degenerated glands. Note higher amount of zones of degenerated glands in short-term BTA-treated animals (**A**; n = 3), whereas short-term controls (**B**; n = 3), long-term controls (**D**; n = 2), and long-term BTA-treated experimental animals (**C**; n = 2) show low levels of degeneration.

a number of different phenomena, among them nuclear DNA fragmentation. DNA fragmentation can be detected in situ by incorporating labeled nucleotides onto the 3' OH ends of the fragments with terminal deoxynucleotidyl transferase enzyme (TdT) and then performing detection of the labeled molecules.[10] TUNEL staining specifically detects this DNA fragmentation. It was performed with a commercially available kit (ApopTag Fluorescein Kit, Intergen, Purchase, New York). Detected fluorescence represents apoptotic nuclei.

RESULTS

The PAS-stained sections of the maxillary sinus turbinates of the control animals of the short- and long-term groups revealed mostly normal mucosal tissue (Fig 2A), with very few glands showing signs of degeneration (see Fig 3B,D for quantitative analysis). The PAS-stained sections of the BTA-treated animals of the short-term group revealed a degeneration or lysis of the submucosal glands (Fig 2B). Especially, glands located close to the turbinate bone were affected. The gland ducts showed atrophy. No cellular infiltration was apparent around the glands. Sections of the contralateral *right* maxillary sinus turbinate showed no difference concerning the glandular degeneration as compared to controls. The histologic picture of the maxillary sinus turbinates of sections of BTA-treated animals of the long-term group revealed far fewer changes in the glands and ducts (Fig 2C). The vessels, bones, and respiratory epithelium appeared unchanged in all groups.

The TUNEL staining of sections of control animals (short- and long-term groups) revealed no apoptosis (Fig 4A). The TUNEL-stained sections of tissue of BTA-treated animals of the short-term group revealed fluorescence in the nuclei all over the tissue, but predominantly in the nuclei of the acini of the submucosal glands and the gland ducts (Fig 4B). The BTA-treated tissues of the animals of the long-term group did not show apoptosis (Fig 4C). Figure 4D demonstrates a negative control of the TUNEL staining performed without TdT, showing that the nuclei are not stained at all. The results of the semiautomatic image analysis support the histologic picture described above and are demonstrated in Fig 3.

DISCUSSION

Submucosal glands are innervated mainly by parasympathetic nerves, but sympathetic structures have also been described.[11] In addition, neuropeptides such as vasoactive intestinal peptide (VIP), calcitonin gene-related peptide (CGRP), substance P (SP), and nitric oxide (NO) are present in nerves around glands.[11-13] Vascular tone is controlled mainly by sympathetic nerves.[14,15] Postganglionic sympathetic nerves join the preganglionic nerves and form the vidian nerve. These nerve fibers supply vascular smooth muscle and mainly regulate nasal airway resistance or patency.[16]

A disturbance in one or several of these systems can lead to hypersecretion of the nasal mucosa, making this disorder a frequent problem that strongly affects patients.

After nasal application of BTA, we could demon-

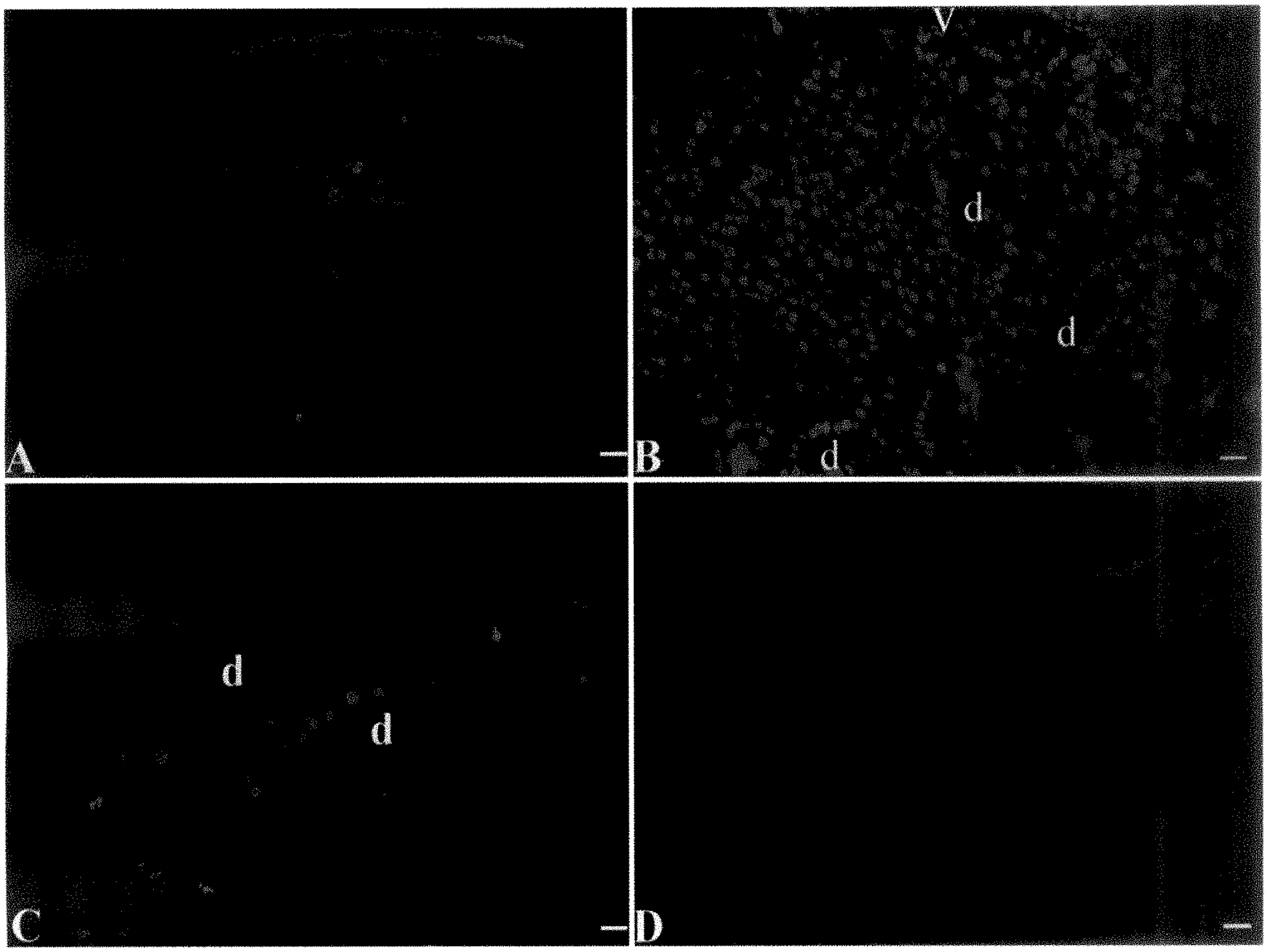

Fig 4. Staining with terminal deoxynucleotidyl-mediated deoxyuridine triphosphate nick end labeling (TUNEL). **A)** TUNEL staining of maxillary sinus turbinate of control animal. Only very few apoptotic nuclei in respiratory epithelium. No fluorescence in glands or gland ducts. Scale bar — 40 µm. **B)** In TUNEL staining of maxillary sinus turbinate of short-term BTA-treated animal, strong fluorescence of apoptotic nuclei was seen in glands (g) and gland ducts (d). v — Vessel; scale bar — 20 µm. **C)** TUNEL staining of maxillary sinus turbinate of long-term BTA-treated animal. Very few apoptotic nuclei are recognizable. d — Duct; scale bar — 20 µm. **D)** Negative control of TUNEL staining. Fluorescent nuclei are not apparent in tissue. Scale bar — 40 µm.

strate a degeneration of nasal glands and ducts. This correlates with the results of the TUNEL stain, which showed diffuse glandular apoptosis. Quantitative analysis confirmed the histologic changes by demonstrating an increased amount of degenerated glands in the BTA-treated short-term group. Since BTA has a cholinergic receptor predilection in its action on nerve terminals, the findings were consistent with localization predominantly in the glandular compartment. These results are in accordance with the findings of Doggweiler et al,[17] who observed generalized atrophy and diffuse glandular apoptosis of prostates of rats after injection of BTA. We did not find any cellular infiltration around the glands. The absence of necrosis or inflammation after exposure to BTA has been described before.[17,18]

In order to be able to make some statement about the duration of the effect of BTA on the nasal submucosal glands, we investigated sections of the maxillary sinus turbinate of both the short-term and long-term groups.

The histologic observations revealed only mild changes of the glandular compartment in the long-term group of treated animals and no apoptosis over that seen in the control group. Since only 2 animals in the long-term group were investigated, we can only report that the effect of BTA on nasal submucosal glands seems to correlate with the observed clinical effect in patients with intrinsic rhinitis, for whom a significant reduction of rhinorrhea lasted about 4 weeks.[8] Further experimental efforts with more animals must be made to confirm these results. The duration of the reduction of rhinorrhea after local application of BTA differs from that after muscular injections (18.2 weeks[19]), and the duration of the effect on the autonomic nervous system also differs (eg, 6 months for lacrimal glands[20] and 17.3 months for sweat glands in gustatory sweating[3,4]). This fact may be explained by the existence of an abundance of blood vessels in the nasal cavity, which causes the absorption and clearance to be more rapid.[8] Botulinum toxin type D is known to be more potent in the autonomic nervous system[9] and might be used therapeutically in the future.

At the beginning of this study, we had to decide on a dosage and mode of application of BTA. Kim et al[8] reported a significant reduction in rhinorrhea in patients with intrinsic rhinitis after injection of 4 units of Botox into the middle and inferior turbinates. In dogs, Shaari et al[9] used gauze soaked in 50 units of Botox. For the nasal cavity size and anatomy of guinea pigs, gauze soaked with 20 units of BTA seemed to be a viable dose and a minimally invasive method to obtain an effect without causing side effects. The applied sponge (Merocel) expands many-fold when soaked and fills the whole nasal cavity with optimal contact with the maxillary sinus turbinate.

The existence of a partial glandular response to BTA treatment might be related to the dose and the method of toxin application and to the fact that many neurotransmitters that are not influenced by the toxin are known to influence the glandular secretory mechanisms.

In summary, it has been shown that BTA induces apoptosis in the nasal glandular compartment of adult guinea pigs. This degeneration was observed 10 days after treatment, but no longer existed in animals sacrificed after 3 months. These findings are consistent with previous clinical observations made in patients with intrinsic rhinitis treated with BTA,[8] in whom rhinorrhea was significantly reduced. Further experimental and clinical work will show whether BTA might be a good therapeutic option for the treatment of rhinorrhea of different causes.

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DEEP NECK ABSCESS: A RETROSPECTIVE REVIEW OF 210 CASES

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This study was performed to review our experience with deep neck abscesses (DNAs) and compare it to the experiences in the available literature, and to study changing trends within our patient population. We retrospectively studied 210 patients who had DNAs between 1981 and 1998. Peritonsillar abscesses and limited intraoral abscesses were excluded. Demographics, presentation, etiology, site of abscess, associated systemic diseases, bacteriology, radiology, treatment, airway management, and outcome were reviewed. We compared the entire group to those in the available literature and studied changing trends within this patient population. Dental infection (43%) was the most common cause, followed by intravenous drug abuse (12%) and pharyngotonsillitis (6%). The incidences of intravenous drug abuse and mandibular fractures as causes of DNA were 19% and 8%, respectively, during the period 1981 to 1990, but were only 1% each during the period 1991 to 1998. *Streptococcus viridans* was the most common pathogen (39% of positive cultures), followed by *Staphylococcus epidermidis* (22%) and *Staphylococcus aureus* (22%). Lateral pharyngeal space abscess was the most common DNA (43%), followed by submandibular space abscess, Ludwig's angina, and retropharyngeal space abscess (28%, 17%, and 12%, respectively). Seventy-five percent of patients with true Ludwig's angina underwent tracheotomy. Nondental infections are no longer a significant etiologic factor in DNA. *Streptococcus viridans* has replaced *S aureus* and β-hemolytic streptococci as the most common pathogen. Lateral pharyngeal space abscess was the most common DNA; however, its incidence has progressively decreased over the past decade. Intravenous drug abuse and mandibular fractures are no longer major etiologic factors. Tracheotomy is indicated in patients with Ludwig's angina.

KEY WORDS — dental infection, Ludwig's angina, neck abscess, retropharyngeal space abscess, submandibular space abscess.

INTRODUCTION

Deep neck abscesses (DNAs) are significantly less common today than in the past. The impact of antibiotic treatment and improved dental care are the most probable reasons for this change. However, studies have shown that violence, substance abuse, and lack of proper primary medical and dental care have made DNA a relatively frequent entity in inner city populations. Recent studies have also demonstrated changing trends in the causes, bacteriologic findings, diagnosis, and management of DNAs.[1-4]

MATERIALS AND METHODS

We retrospectively reviewed the records of patients with the diagnosis of neck abscess who were seen at Kings County Medical Center between 1981 and 1998. Patients with peritonsillar abscess, superficial skin abscess, limited-space intraoral abscess, and cervical necrotizing fasciitis were excluded. Two hundred ten charts that met the above-mentioned criteria were reviewed. Clinical charts, as well as radiologic and bacteriologic studies, were reviewed. Demographic data, symptoms and their duration, radiographic tests performed and their results, dates and types of procedures, interventions for airway management, type and duration of antibiotic treatment, results of cultures, and duration of hospital stay were also recorded. The abscesses were divided into 4 anatomic groups: submandibular space, lateral pharyngeal space, retropharyngeal space, and Ludwig's angina. The findings were compared to those in the available literature. Changing trends within the group were also studied by comparing our findings from the period 1981 to 1990[4] with those from the period 1991 to 1998.

RESULTS

Two hundred ten charts were reviewed; the patients were 119 men (57%) and 91 women (43%). The age distribution curve is shown in Fig 1. Almost 50% of the patients were in their third or fourth decade of life. One hundred fifty-six patients (74%) were African-American, 33 patients (16%) were His-

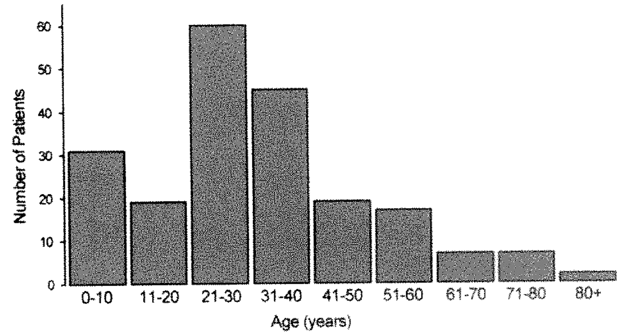

Fig 1. Age distribution of 210 patients with deep neck abscesses.

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TABLE 1. CAUSES OF ABSCESSES

Cause	*No.*	*%**
Dental	77	43
Intravenous drug abuse	21	12
Pharyngotonsillitis	12	6.7
Mandibular fracture	10	5.6
Skin infection	9	5.1
Tuberculosis	9	5.1
Foreign body	7	3.9
Trauma	6	3.4
Peritonsillar abscess	6	3.4
Sialolithiasis	5	2.8
Parotitis	3	1.7
Miscellaneous (branchiogenic, laryngopyocele, Bezold's abscess, Kenalog injection)	10	5.6
Unknown	35	

*Of 175 cases with known causes.

panic, 19 patients (9%) were white, and 2 patients (1%) were Asian. This ethnic distribution is representative of central Brooklyn.

The cause of the abscess was identified in 175 patients (83.3%; Table 1). The most common cause was dental infection (43%). Dental infection was the cause in 76% of Ludwig's angina abscesses and 61% of submandibular space abscesses. Dental infection was not the known cause in any of the retropharyngeal space abscesses.

The second most common cause of DNA was intravenous drug abuse (IVDA; 12%). Of 21 cases attributed directly to IVDA, 20 occurred between 1981 and 1990, and only 1 case was found between 1991 and 1998. In the period 1981 to 1990, mandibular fractures were the cause of 9 abscesses; however, there was only 1 abscess in the period 1991 to 1998 that was attributable to mandibular fracture. Pharyngotonsillitis was the known cause in 6% of patients. Other causes of DNAs are listed in Table 1.

Diabetes mellitus was the most common associated systemic disease, occurring in 34 patients (16%). Fifteen patients (7%) had positive human immunodeficiency virus (HIV) status, and 17 patients (8%) had a diagnosis of acquired immunodeficiency syndrome (AIDS). Two patients (1%) had sickle cell disease.

The mean white blood cell (WBC) count in the study was 15,500 cells per cubic millimeter, with a range of 2,100 to 45,900 cells per cubic millimeter. Overall, 32 patients (15%) had WBC counts of less than 8,000 cells per cubic millimeter, of whom 19 (59%) had a diagnosis of AIDS or HIV. The mean WBC count for patients with HIV or AIDS was 9,000 cells per cubic millimeter.

Results of bacterial cultures were available in 186 patients (88%). Of them, 162 (87%) had bacterial growth and 24 (13%) had no growth. Sixty-two percent of positive cultures were polymicrobial. The most common bacterial organism was *Streptococcus viridans* (39%), followed by *Staphylococcus epidermidis* (21%). The single most common anaerobe was *Bacteroides* species (14%). Anaerobes constituted 35% of the overall bacterial isolates. Gram-negative aerobic organisms such as *Proteus*, *Pseudomonas*, and *Escherichia coli* were found in fewer than 4% of bacterial isolates (Table 2).

TABLE 2. BACTERIAL CAUSES OF DEEP NECK ABSCESSES

Organism	*No. of Patients*	*Percent of Positive Cultures*
Streptococcus viridans	63	39
Staphylococcus epidermidis	46	28
Staphylococcus aureus	35	22
Bacteroides spp	22	14
β-Hemolytic streptococci	34	21
Non-A, -B, or -D	12	7.4
Type A	9	5.5
Type B	13	8.0
Klebsiella pneumoniae	11	6.8
Streptococcus pneumoniae	10	6.2
Mycobacterium tuberculosis	10	6.2
Anaerobic gram-negative rods	9	5.5
Neisseria spp	8	4.9
Peptostreptococcus	8	4.9
Yeasts	8	4.9
Enterobacter	7	4.3
Bacillus spp	6	3.7
Propionibacterium	6	3.7
Acinetobacter	5	3.1
Anaerobic gram-negative cocci	4	2.5
Actinomycosis israelii	3	1.9
Proteus spp	3	1.9
Other *Klebsiella* spp	3	1.9
Bifidobacterium	3	1.9
Microaerophilic streptococci	3	1.9
Enterococcus spp	3	1.9
Moraxella catarrhalis	2	1.2
Citrobacter	1	0.6
Diphtheroides	1	0.6
Salmonella	1	0.6
Fusobacterium	1	0.6

The lateral pharyngeal space abscess was the most common abscess (43%). Submandibular space, Ludwig's, and retropharyngeal space abscesses were found in 28%, 17%, and 12% of patients, respectively. Lateral pharyngeal space abscesses constituted 50% of the DNAs from 1981 to 1991; however, their incidence decreased to about 30% of the DNAs between 1991 and 1998.

TABLE 3. DISTRIBUTION OF CASES BY TIME PERIOD

	1981-1990		*1991-1998*	
Abscess Location	*No.*	*%*	*No.*	*%*
Submandibular space	23	21	36	36
Lateral pharyngeal space	60	54	30	30
Retropharyngeal space	12	11	13	13
Ludwig's abscess	15	14	21	21

Between 1991 and 1998, contrast-enhanced computed tomography (CT) was used to confirm the clinical diagnosis in all retropharyngeal space abscesses and in 97% of lateral pharyngeal space abscesses. Computed tomography was used to confirm the clinical diagnosis in submandibular space and Ludwig's abscesses in fewer than 20% of cases.

The incidence of tracheotomy was reviewed for all abscess types (Table 3). Of patients with submandibular space, lateral pharyngeal space, and retropharyngeal space abscesses, 1.7%, 7.8%, and 25% required tracheotomy, respectively. Of 36 patients with Ludwig's angina, 16 underwent elective awake tracheotomy under local anesthesia on admission. Endotracheal intubation was attempted in the remaining 20 patients. Intubation failed in 11 of these patients (55%), and they required emergency ("slash") tracheotomy. The overall incidence of tracheotomy in patients with Ludwig's angina was 75% (Fig 2).

Of 210 patients, 205 (97.6%) were discharged without significant complications. The mean abscess-related hospital stay was 9.5 days. There was 1 death from aspiration pneumonia, 2 cases of mediastinitis, 1 case of cervical discitis, and 1 case of injury to the innominate artery during a "slash" tracheotomy. Immediate thoracotomy with repair of the innominate artery was performed. The patient was discharged in 20 days without significant sequelae.

DISCUSSION

The DNAs occurred in a wide age range (Fig 1). However, 50% of the patients were in their third or fourth decade, probably because of the relatively high rates of dental infection and IVDA in this age group. The ethnic distribution of DNA patients in this study was consistent with that of central Brooklyn.

In the preantibiotic era, pharyngotonsillitis was the cause in nearly 70% of DNAs, whereas dental infection was the cause in only 20% of DNAs.[5] In our series, 43% of DNA cases in which the cause was known were secondary to dental infection, and only 6% of cases with a known cause were attributable to pharyngotonsillitis. This decline in the role of pharyngotonsillitis is generally attributable to the widespread use of antibiotics.

Lateral pharyngeal space abscess was the most common DNA, followed by submandibular, Ludwig's, and retropharyngeal abscesses. In our series, a significant decline in the incidence of lateral pharyngeal space abscesses was observed. Lateral pharyngeal space abscesses constituted nearly 50% of all DNAs between 1981 and 1990. However, between 1991 and 1998, they constituted only 30% of all DNAs (Table 3). Dental infections leading to cervical lymphadenitis and subsequent abscess formation were still the most common cause of lateral pharyngeal space abscesses. The incidence of IVDA as an etiologic factor for DNA decreased nearly 20-fold from the 1980s to the 1990s. As abscesses caused by IVDA are usually in the lateral pharyngeal space, the decrease in this DNA site may be explained by a decrease in IVDA in central Brooklyn, or by a changing pattern of IVDA (for example, a decrease in intravenous use of crack cocaine).[6]

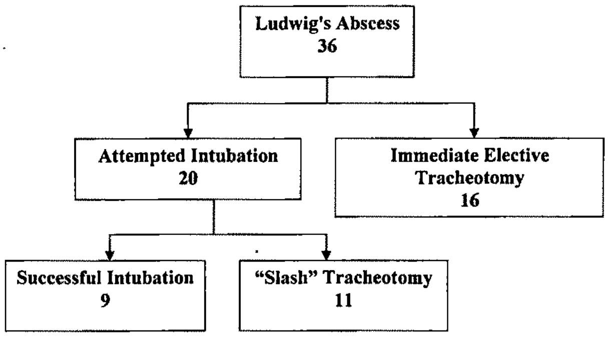

Fig 2. Incidence of tracheotomy in Ludwig's angina (abscess).

A decrease in violence in central Brooklyn has led to a significant decrease in the incidence of mandibular fractures over the past decade and, hence, the decrease in the incidence of mandibular fractures as a cause of DNA: from 9% in the period 1981 to 1990 to 1% in the period 1991 to 1998.

One third of our patients with DNAs also had an associated systemic disorder. Although our study did not demonstrate different causes or bacteriologic findings in patients with AIDS, other studies have shown higher rates of resistant organisms in these patients.[7]

Two thirds of our bacterial cultures were polymicrobial — a finding consistent with that of previous publications.[8] The most commonly isolated organism was *S viridans* (39%), consistent with the high rate of cases of dental origin. Thirty-five percent of cultures grew anaerobes; this is possibly an underestimation of the true role of anaerobes in DNAs due to widespread antibiotic use prior to collection of cultures, poor sample collection techniques, and the fragility of these organisms. The decreased role of β-hemolytic streptococci in DNAs is consistent with

TABLE 4. INCIDENCE OF TRACHEOTOMY

Abscess Location	*No. of Patients*	*%*
Submandibular space	1/59	1.7
Lateral pharyngeal space	7/90	7.8
Retropharyngeal space	8/25	32
Ludwig's abscess	27/36	75

the decreased incidence of pharyngotonsillitis as an etiologic factor in DNAs.

We did not detect a high rate of resistant organisms in our series, and on the basis of the bacteriologic findings, we believe that penicillin G with oxacillin or nafcillin will provide adequate initial coverage in most patients. Since aerobic gram-negative organisms were uncommon in our study, we do not recommend the use of additional antibiotics such as gentamicin as part of the initial management of DNA. Local resistance patterns, as well as the immunocompetence of the patient, may alter the choice of first-line antibiotic agent.

Contrast-enhanced CT scanning was used to confirm the diagnosis and to assess the extent of the abscess in all cases of retropharyngeal space abscess and in most cases of lateral pharyngeal space abscess. A CT scan is not essential to confirm the diagnosis of submandibular space abscess or Ludwig's angina.

All patients with lateral pharyngeal space, submandibular space, and Ludwig's abscesses underwent external incision and drainage. Although needle aspiration and intravenous antibiotics may be adequate treatment in selected cases,[9] we continue to use incision and drainage as the standard treatment of choice.

Retropharyngeal space abscesses that did not involve other deep neck spaces were treated with transoral incision and drainage. Careful attention to airway management is paramount in retropharyngeal space abscess management, because rupture of the abscess by the endotracheal tube may result in spillage and aspiration pneumonia.

Airway management is the first step in management of patients with DNAs. We reviewed the role of tracheotomy in airway management in our patients (Table 4). Tracheotomy was rarely performed in patients with submandibular space abscesses. The role of tracheotomy was clearly significant in patients with Ludwig's angina. We defined Ludwig's angina as edema of the entire floor of the mouth. The edema of the floor of the mouth pushes the tongue upward and backward, obstructing the oropharynx and causing significant airway distress. These patients cannot tolerate their own saliva. In our series, 44% of patients with Ludwig's angina underwent uneventful awake elective tracheotomy before incision and drainage (Fig 2). Endotracheal intubation was attempted in the remaining 56%. However, it was unsuccessful in 55% of those cases. Acute deterioration in respiratory status resulted in emergency, "slash" tracheotomy in these patients. These findings have convinced us that elective awake tracheotomy is a safer method of airway management in patients with a fully developed Ludwig's abscess.

In DNA patients from the past 18 years, we have seen 1 death as a result of aspiration pneumonia, and no major vessel rupture. The combination of early radiologic diagnosis, effective antibiotic therapy, and intensive care management has contributed to a good prognosis in patients with DNA.

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VENOUS COLLATERAL BLOOD FLOW ASSESSED BY DOPPLER ULTRASOUND AFTER UNILATERAL RADICAL NECK DISSECTION

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Removal of the internal jugular vein (IJV) in unilateral radical neck dissection (rND) necessitates redirection of cerebrovenous blood to collateral pathways. If adaptation is insufficient, neurologic sequelae develop that are due to impaired venous drainage and increased intracranial pressure. The authors studied venous hemodynamic effects of unilateral rND using Doppler and duplex ultrasound in 17 patients. Blood flow velocities (BFVs) were recorded from the distal IJV (dIJV) and the vertebral vein (VV) before and 9 to 88 days after surgery. A preoperative compression test of the dIJV was performed to identify the side of dominant drainage. The BFV increased in the contralateral dIJV after right-sided rND (n = 10) by 111% (range, 50% to 320%), and after left-sided rND (n = 7) by 34% (range, 5% to 105%). In the contralateral VV, a rise of BFV by 75% was found. Our results confirm the role of the contralateral dIJV as the predominant collateral pathway. The VVs serve as an important additional major outflow. Doppler ultrasound may help to identify patients at risk of insufficient cerebrovenous drainage after rND.

KEY WORDS — cerebral venous system, Doppler sonography, internal jugular vein, radical neck dissection, vertebral vein.

INTRODUCTION

Unilateral radical neck dissection (rND) involves resection of the internal jugular vein (IJV) and external jugular vein, including their tributaries from the neck, the sternocleidomastoid muscle, and the adjacent lymphatic tissue on one side of the neck.[1] The IJVs are thought to be the most important pathway for venous blood returning from the brain. The observation that even bilateral rND is usually well tolerated indicates the presence of alternative pathways that allow sufficient redirection of cerebrovenous blood.[2] On the other hand, substantially elevated intracranial pressure is encountered in a minority of patients after rND and may lead to a fatal outcome. Until now, no systematic studies investigating the collateral pathways that exist after rND have been performed.

SUBJECTS AND METHODS

Seventeen patients (14 men and 3 women; mean age ± SD, 57.5 ± 7.4 years) with cervical malignancies were examined before and after surgery (range, 9 to 88 days; median, 17 days). The patency of the distal IJV (dIJV) was checked before rND by means of B-mode and duplex imaging. Patients revealing compression of the dIJV by tumor mass were excluded from the analysis. The rND was carried out on the right side in 10 patients and on the left side in 7. The mean blood flow velocities (BFVs; Vmean) were measured in the dIJV with a range-gated 2-MHz transducer (Multidop-X, DWL), and systolic BFVs were measured in the vertebral vein (VV) with a 5-MHz linear transducer (Sonolayer SSA-270A, Toshiba) with the patient in the supine position.[3,4] Additionally, BFV changes occurring in the dIJV during short manual compression of the opposite IJV were used to identify the dominant IJV before rND. After rND, clinical signs of intracranial hypertension were evaluated. For statistical evaluation, Wilcoxon signed rank tests were performed for paired samples, and Mann-Whitney tests were used for unpaired samples. A value of $p < .05$ was considered significant.

For statistical evaluation of the VV, preoperative and postoperative flow velocities were grouped into 4 categories. Peak flow velocities from 0 (not detectable) to 11 cm/s were rated as I, 12 to 23 as II, 24 to 35 as III, and 36 to 47 as IV, and peak flow velocities of 48 cm/s or more were rated as V. This approach was chosen because, in our opinion, the absence of a detectable signal was related to a low-volume flow, rather than reflecting an actual zero flow.

RESULTS

In the dIJV, baseline mean (±SD) flow velocities of 25.6 ± 9.4 cm/s (right) and 23.4 ± 9.5 cm/s (left; p not significant) were measured. A marked increase of Vmean in the contralateral dIJV was recorded in all patients after unilateral rND ($p < .001$). The post-

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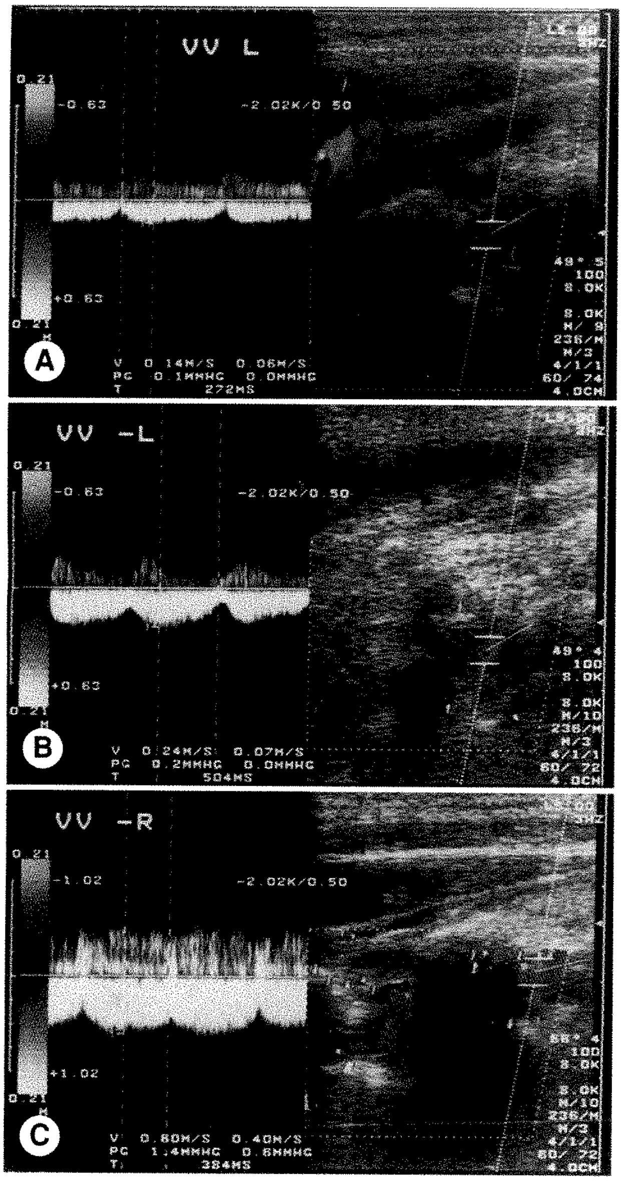

Color-coded duplex sonograms of vertebral veins. **A**) Before left-sided radical neck dissection, systolic/diastolic blood flow velocities were 14/6 cm/s in left vertebral vein. **B**) Twenty-four days after surgery, velocities were 24/7 in left vertebral vein. Initially, no blood flow was found in right vertebral vein (not shown). **C**) After neck dissection, high velocities of 60/40 cm/s were present in right vertebral vein.

operative increase was higher than the increase during the preoperative compression test (p < .001). Furthermore, the increase of BFV in the contralateral dIJV was higher after right-sided rND (p = .029). After right-sided surgery, the increase of BFV in the left dIJV ranged from 50% to 320% of the preoperative baseline (mean, 111%; p = .012). In the preoperative assessment by manual compression of the right dIJV, a smaller increase of up to 150% (mean, 42%) was observed (difference between surgery and compression test, p = .043). After left-sided surgery, the increase in the right dIJV ranged from 5% to 105% (mean, 34%; p = .018), compared with an increase up to 66% (mean, 17%) during contralateral compression of the left dIJV (p = .109).

The VV was examined in 14 of 17 patients. After rND, there was a significant increase of systolic BFV in both VVs taken together (p = .008). After right-sided rND (n = 8), an increase of systolic BFV was seen in the left VV in 7 cases (88%; p = .024). In 4 of these patients, a systolic flow signal (mean systolic BFV, 26.7 cm/s) was demonstrated only after rND. In the remaining 3 cases, a mean increase of systolic BFV by 99.3% was found. No significant changes were seen in the ipsilateral VV after right-sided rND (p = .71).

After left-sided rND (n = 6), a pronounced rise of systolic BFV was observed in the contralateral VV in 5 patients (83%; p = .11) and in the ipsilateral VV in 4 patients (67%; p = .10). Flow signals could not be detected before rND in the contralateral VV in 4 of these patients or in the ipsilateral VV in 2 of these patients (see Figure).

Two patients developed transient neurologic symptoms after rND. A 58-year-old patient displayed basal flow velocities of 59 cm/s in the right dIJV and 18 cm/s in the left dIJV. After right-sided rND, the flow velocity increased to 42 cm/s (133%) in the left dIJV. Neither VV was detected before rND, but after rND, systolic flow velocities of 22 cm/s (right) and 13 cm/s (left) were measured. This patient complained of severe headache and diminished visual acuity that persisted for 34 days. Similar findings were encountered in another man, 54 years old. In both cases, the neurologic symptoms resolved completely before discharge.

DISCUSSION

The IJVs are the main drainage vessels for cerebral venous blood.[5] In addition, up to 20% of the flow in the external jugular vein — which is also removed in rND — originates from the brain.[6] Because of the perceived relevance of the external jugular vein as a potential venous collateral after rND, a modified technique with preservation of this vessel was suggested.[7] While a severe increase of intracranial pressure is rare even after bilateral rND,[2,8] intracranial hypertension after unilateral rND was reported to occur 8 times more frequently after right-sided rND.[9] This finding reflects the well-known right-sided dominance of the venous drainage of the brain.[10] Intracranial hypertension may indicate unilateral drainage of the superior sagittal sinus and straight sinus to one IJV, which has been reported in 4% to 9% of cases.[11,12] Also, in our study, a more pronounced contralateral increase of BFV in the dIJV was observed after right-sided rND, and 2 patients who had right-sided rND had clinical symptoms of moderately increased intracranial pressure.

In our study, the compression test confirmed the right-sided dominance of the cerebrovenous drainage.[3,10] However, the BFV increase in the contralateral dIJV was far more pronounced after rND than during compression — a finding that may reflect collateral flow through the external jugular vein and its tributaries. The importance of these vessels is confirmed further by the sharp rise of intracranial pressure that is induced by compression of the dorsal neck region in patients after bilateral rND.[8]

Obvious changes in BFV were observed in the VVs after rND. The VV is a major collecting vessel of the vertebral venous system, which probably has sufficient capacity to take over the entire venous drainage of the brain.[2,12] Angiographic studies argue that in monkeys, the vertebral venous system is the main outflow pathway in the upright position, whereas the jugular veins are found to collapse.[13] In a duplex study of the postural dependency of the cerebral venous outflow in humans, a gradual collapse of the dIJV was demonstrated starting at +15° that was paralleled by a marked rise of flow in the VVs.[14] Other authors found a significantly larger cross-sectional area of the dIJV in the supine position than in the upright position on both sides.[15] This finding may explain the clinical observation that many patients do not tolerate the horizontal position during the first few days after rND.[1,2,16] Long-lasting headaches and other symptoms of increased intracranial pressure that occur when the patient is lying flat are frequently described in the literature.[1] In our series, 2 patients suffered from severe headache. In both individuals, a substantial increase of mean flow velocity in the contralateral dIJV and of systolic flow velocity in both VVs was detected. The resection of the dominant IJV and the following dramatic increase of contralateral flow velocities might be the reason for the severe clinical picture. However, other patients with rND on the dominant side, as well as patients with higher relative flow increases, were asymptomatic after rND.

The time that collaterals need to develop is not exactly known. Periods ranging from a few weeks to many months have been described.[17] Apparently, some degree of adaptation occurs within a few minutes after ligation of the IVJ, probably by redirection of the venous blood through preexisting collateral pathways. Clinical signs of elevated intracranial pressure may, however, last for some weeks,[2] and elevated cerebrospinal fluid pressure is measured for several days after surgery, suggesting a long-term adaptation within months.[8,14,18-21] It is generally believed that bilateral rND is a safer procedure when the neck dissections are staged with an interval of at least 1 month to allow the development of sufficient collateral venous drainage from the central nervous system.[22] Therefore, the BFVs measured in our study might change before further examinations.

The presented study shows that ultrasound techniques allow the assessment of venous hemodynamic changes following rND. Doppler studies may provide important functional information in addition to the visualization of vessel diameters on preoperative magnetic resonance images.

The most important collateral pathway for the redirection of cerebrovenous blood is the contralateral IJV, followed in importance by the VVs. Consistent with the well-known side difference of the cerebral venous outflow, ligation of the dominant dIJV causes much more pronounced changes of the venous drainage. Preoperative Doppler ultrasound including compression tests may be used to identify the dominant side of venous drainage and may — especially before bilateral rND or in the rare case of completely unilateral drainage — allow assessment of the risk of postoperative intracranial hypertension.

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ACCOMMODATION TO CHANGES IN BOLUS VISCOSITY IN NORMAL DEGLUTITION: A VIDEOFLUOROSCOPIC STUDY

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Videofluoroscopic swallow studies were performed on 60 normal adult volunteers. Swallowing variables were measured during swallows of a 3-cm^3 paste bolus and a 3-mL liquid bolus and were compared to identify changes in swallow gesture displacement and timing, as well as changes in bolus movement timing. The study revealed that some differences measured by videofluoroscopy appear to be the result of the inherent bolus characteristics, while others likely represent changes in swallow gestures needed to accommodate variations in bolus viscosity. The overall timing of pharyngeal transit did not vary between bolus types. Oropharyngeal transit trended toward being faster for a liquid bolus with a concurrent early elevation of the aryepiglottic folds. The hyoid bone elevated at the same time, at the same rate, and to the same extent irrespective of bolus viscosity. During a liquid bolus swallow, the hyoid bone trended toward a more prolonged elevation, corresponding to prolonged pharyngoesophageal sphincter opening. The pharyngoesophageal sphincter, however, opened to a greater extent with a paste bolus.

KEY WORDS — bolus viscosity, deglutition, swallowing.

INTRODUCTION

Videofluoroscopy is a well-established clinical tool for the evaluation of swallowing in dysphagic patients. The effective interpretation of videofluoroscopic findings depends on the availability of objective and reproducible normative data with which to compare videofluoroscopic findings in patients with dysphagia. The purpose of this study was to establish a set of normative swallowing data allowing the analysis of viscous bolus deglutition, and to evaluate accommodations made in order to handle the more viscous bolus type. This study reports the results of dynamic videofluoroscopic swallowing studies performed in 60 normal adult volunteers. Swallowing gesture timing and bolus transit timing were measured during a 3-mL liquid bolus swallow and a 3-cm^3 paste bolus swallow. The coordination of swallowing gestures with bolus transit was analyzed, and the effects of changes in bolus consistency both on swallow gesture timing and extent and on bolus transit were identified. The evaluation of swallow gesture coordination relative to bolus transit enables us to identify cases in which gesture timing is altered as a result of disease or used as a strategy to overcome functional abnormalities. By plotting timing measurements in dysphagic patients relative to normal values, we can easily identify a visual depiction of variations from the norm (see Figure).

During swallowing, the human upper aerodigestive tract responds to changes in bolus volume and consistency in order to maintain efficient transit of the bolus from the oral cavity to the upper esophagus. The coordination and force of the swallow gestures can be altered according to the bolus characteristics. The impact of those changes in pharyngeal muscle activity can be determined by the analysis of bolus transit through the pharynx. Although many previous evaluations of swallowing in normal subjects have documented differences in oropharyngeal muscular activity and subsequent pressure changes with various bolus viscosities, few have reported on changes in the timing and extent of swallow gestures as they relate directly to their impact on bolus transit. For example, one might expect to see differences in hyoid bone elevation and pharyngeal constriction with changes in bolus viscosity, as both are known to be involved in developing the forces necessary to drive a bolus into the upper esophagus.

The most comprehensive evaluation of swallowing variables includes simultaneous manometric, videofluoroscopic, and electromyographic measurements. Data provided by this sort of study include bolus transit timing, swallow gesture timing, pharyngeal pressure generation, and the force of oropharyngeal muscular contractions. All of these factors no doubt play a role in the accommodation of a bolus during swallowing. In a clinical setting, however, this type of in-depth analysis may not be available. In addition, measured swallowing variables are often defined differently, depending on the preference of

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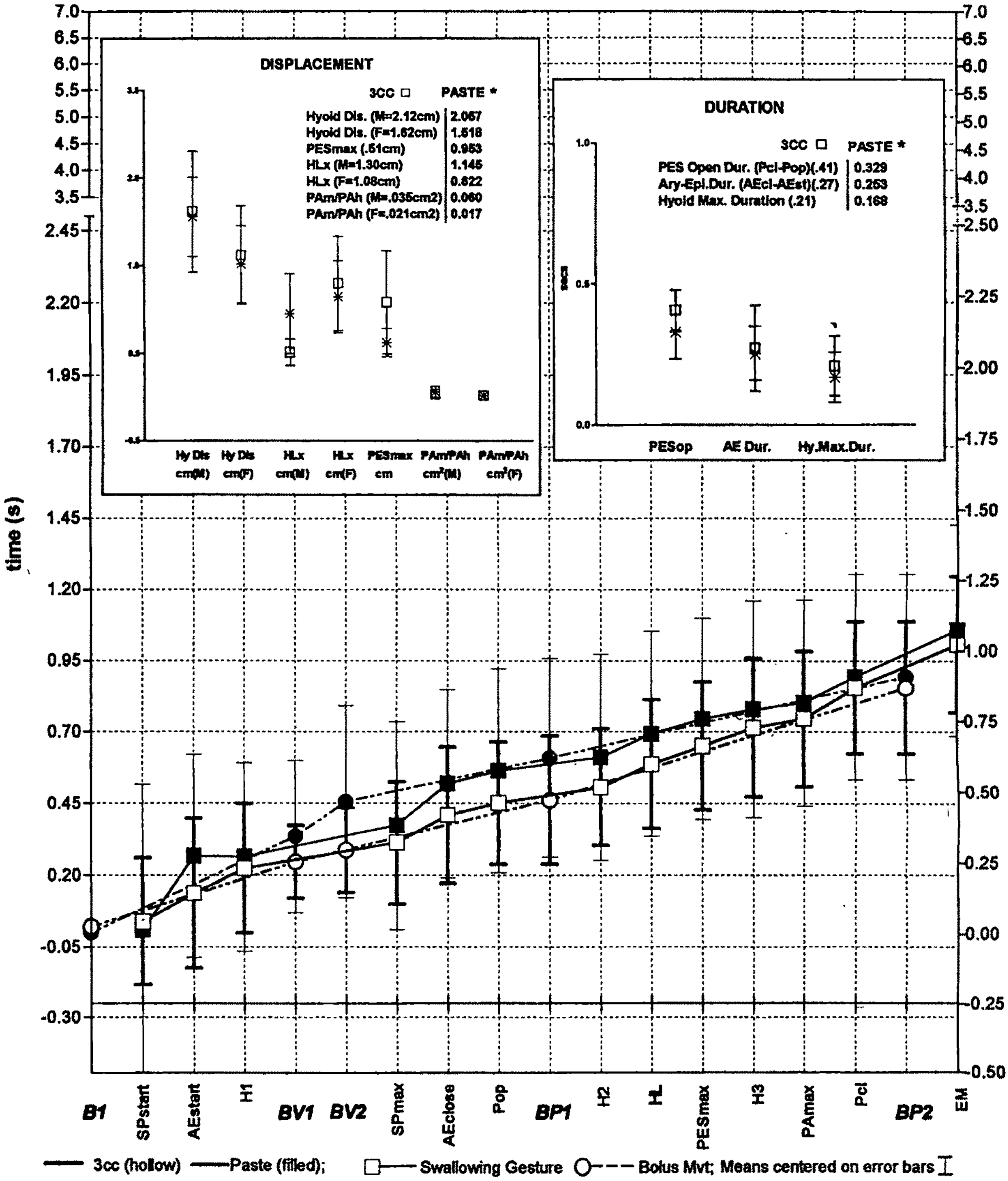

BOLUS: B1-bolus head passes posterior nasal spine; BV1-enters valleculae; BV2-exits valleculae; BP1-enters PES; BP2-clears PES

GESTURES: SPstart-soft palate starts elevation; AEstart-arytenoid-epiglottis (larynx close) start; H1- first hyoid displacement; SPmax -soft palate reaches max. elevation; AEclose-max. arytenoid-epiglottis closure; Pop- PES opens: H2-hyoid max. displacement; HL-max. hyoid-larynx approximation; PESmax.-max. opening; Pcl-PES closes EM-epiglottis returns to upright position.

Graph depicts mean and standard deviation of gesture and bolus timing from 3-mL liquid swallow and 3-cm^3 paste swallow. Boxes contain displacement and duration of displacement data for two swallow types.

the reporting institution, so that direct clinical application of the data is difficult. We have restricted our analysis, therefore, to the type of assessment most readily available in our institution and the community at large, ie, videofluoroscopy.

BACKGROUND

Previous work in this area has usually involved small numbers of subjects and has generated mixed results. In particular, those studies that evaluate the timing of bolus transit report contradictory findings, possibly as a consequence of different assessment methods. Dantas et al[1] evaluated swallowing in 10 healthy volunteers and determined that "pharyngeal clearance time," defined as the time between the movement of the bolus head past the fauces of the tonsils and closure of the pharyngoesophageal sphincter (PES) on videofluoroscopy, was prolonged for the more viscous bolus type. No similar accommodation was identified during swallowing of different bolus volumes. On the other hand, using scintigraphy, Hamlet et al[2] found that pharyngeal transit times were not significantly different with variations in bolus viscosity in 20 normal volunteers. Larger pharyngeal residues, however, were observed for more viscous materials.[2] In a similar study, Kim et al[3] noted that "oropharyngeal emptying" was not influenced by bolus viscosity in 38 healthy volunteers. The present study was designed to determine whether the timing of bolus pharyngeal transit as measured by videofluoroscopy was altered in response to changes in bolus viscosity in a large cohort (n = 60) of subjects.

The study by Dantas et al[1] further identified a prolongation of PES opening with a swallow of paste barium as opposed to liquid barium. In addition, the flow of bolus material through the PES was slower with thicker consistencies. Coinciding with a longer period of PES opening, Dantas et al[1] found that the hyoid bone–larynx complex moved farther anteriorly during swallowing of a paste bolus. Studies that evaluated the activity in the suprahyoid musculature with electromyography confirmed increased strength and duration of contractile activity in those muscles used for elevation of the hyoid bone and larynx when a more viscous bolus was swallowed.[4,5] In this study, these and additional variables will be evaluated relative to the position of the bolus in the pharynx.

Manometric studies that have evaluated swallowing relative to changes in bolus viscosity have uniformly identified an increase in *oral* intraluminal pressures generated during swallowing of thicker materials. Pouderoux and Kahrilas[6] used sensory bulbs and strain gauges to measure tongue pressure forces in 8 volunteers. Increased bolus viscosity induced increases of tongue propulsive force and of clearing pressure by the oral tongue. No force adaptation to an increased bolus volume was found, however.[6] These findings confirm those of Perlman et al,[7] who found that the duration of the pressure exerted by the tongue on the bolus before the generation of pharyngeal pressures was significantly affected by bolus viscosity in 40 adult volunteers. Manometric studies of the pharynx indicate an increase in the duration, but not the amplitude, of pharyngeal peristaltic waves during swallowing of more viscous bolus materials.[1] Whether changes in pharyngeal pressures result in identifiable alterations in pharyngeal swallow gesture timing and/or duration as measured by videofluoroscopy has not been determined.

MATERIALS AND METHODS

SUBJECTS

Dynamic videofluoroscopic swallow studies were performed on 60 consecutive normal adult volunteers. The study group consisted of 30 men and 30 women with an age range of 18 to 73 years. None of the study subjects had any complaints of dysphagia or a history of central nervous system or craniofacial abnormalities.

PROCEDURES

The radiographic studies were conducted in the Voice-Speech-Swallowing Center at the University of California–Davis Medical Center in accordance with the routine radiographic protocols approved by the institution. The equipment used included a properly collimated Phillips radiographic-fluoroscopic unit that provides a 63-kV, 1.2-mA type of output for the full field of view mode (9-inch input phosphor diameter). Fluoroscopy studies were recorded on high-quality videotape for playback and analysis with a Sony model 1380 videocassette VHS recorder-player. A graphic time display provided by an RCA character generator and JVC AC adapter model C412 was included on the videotape so that timing information at 0.01-second intervals was recorded. The swallow studies were recorded at 30 frames per second. Measures were made during the swallowing of a 3-mL liquid bolus (liquid Barosperse barium sulfate suspension, Lafayette Pharmaceuticals, Inc, Anaheim, California) and of a 3-cm^3 paste bolus (Intropaste barium sulfate paste, Lafayette Pharmaceuticals, Inc). The materials to be swallowed were presented to the subject by spoon.

The results from each subject were analyzed by the primary author (K.A.K.) without information about the identity of the subject. The timing measures were reported in seconds. The videotaped images were captured on a Cardio-Loop (Eigen, Inc), which allowed for image enhancement and manipulation when needed. The details of the specific timing measures evaluated are discussed in the next section. From the Cardio-Loop, selected frames were input into a personal computer with a digitizing board from Data Translation, Inc (Marlboro, Massachusetts). The software program used for analysis of fluoroscopic images, called Image, was developed by Wayne Rasband and his colleagues at the National Institutes of Mental Health and is a public domain program (http://rsb.info.nih.gov/nih-image). Area

TABLE 1. ABBREVIATIONS AND DEFINITIONS

B1	First movement of bolus from "hold" position to pass posterior nasal spine
Pcl	Moment pharyngoesophageal sphincter closes and bolus has fully entered esophagus
BV	Arrival of bolus head in vallecula
AEstart	Onset of aryepiglottic fold elevation
AEclose	Aryepiglottic fold elevation to point of supraglottic closure
H1	First superior-anterior movement of hyoid bone
H2	Point at which hyoid bone reaches its maximum displacement
H3	Moment hyoid bone begins its descent to resting position
Pop	Onset of pharyngoesophageal sphincter opening
BP	Arrival of bolus at pharyngoesophageal sphincter
PESmax	Time at which pharyngoesophageal sphincter has reached its widest opening

and distance measurements were made after calibration of the digitized image to the size of the 1.7-cm-diameter wire loop taped to the chin of the study subject.

MEASURES

Table 1 gives a list of the definitions and abbreviations used throughout this article.

BOLUS TRANSIT TIMING

Bolus transit times reflect the location of a bolus in the pharynx at a given moment during a swallow. Bolus pharyngeal transit time is defined as the time between the onset and completion of bolus pharyngeal transit. The onset of pharyngeal transit is designated B1 and is defined as the first movement of the head of the bolus past the posterior nasal spine. The posterior nasal spine is located at the end of the hard palate and is a good landmark for the anterior border of the oropharynx. Oral transit is not included in the measurement of pharyngeal transit time. The completion of pharyngeal transit is defined as the moment the PES closes and the bolus has fully entered the esophagus. Closure of the PES is designated Pcl. Pharyngeal transit time is calculated as Pcl – B1. Pharyngeal transit time can be divided into oropharyngeal and hypopharyngeal phases by the arrival of the bolus in the vallecula, designated BV. If the bolus bypasses the vallecula, the time at which the bolus passes the level of the base of the vallecula is designated BV.

SWALLOW GESTURE TIMING

The aryepiglottic folds and the outline of the arytenoid cartilages are visible against the relative hypodensity of the pharynx in the lateral projection on the radiograph. As a result, it is possible to identify when the arytenoid cartilages begin to elevate and when they make contact with the downfolding epiglottis.[8] The onset of aryepiglottic fold–cartilage elevation relative to the start of the swallow (AEstart – B1) and the time required for aryepiglottic fold–cartilage elevation to the point of supraglottic closure (AEclose – AEstart) are therefore measured.

The hyoid bone moves during a swallow as a result of suprahyoid muscle contraction.[1,4,9,10] The first superior-anterior movement of the hyoid bone that results in a swallow is designated H1. H2 is the point at which the hyoid bone reaches its maximum displacement during the swallow. The instant the hyoid bone begins its descent to a resting position is designated H3. The onset of hyoid elevation relative to the onset of pharyngeal transit (H1 – B1) and the duration of hyoid elevation (H2 – H3) can be calculated.

The onset of PES opening relative to the onset of bolus pharyngeal transit and the duration of PES opening are measured. Pop designates the time at which the PES opens. This time is identified as the moment the narrowest part of the upper esophagus between C4 and C6 enlarges. We feel that this point best defines the location of the PES. It is also reliably identified on a dynamic swallow study, as opposed to being a measurement made from the often poorly defined top of the air column in the trachea.[6,9] PESmax is the time at which the PES has reached its widest opening (in lateral view).

As the bolus is propelled into the upper esophagus, the pharynx is almost completely obliterated by the tongue pushing against the contracting posterior pharyngeal wall. This gesture is crucial to a successful swallow. Poor pharyngeal muscle contraction and contact with the tongue base results in pharyngeal residue that may be aspirated after the swallow.[11,12] The timing of maximum pharyngeal constriction (PAmax) was measured.

SWALLOW GESTURE EXTENT

All measures of gesture extent were obtained from lateral views and included maximal hyoid elevation and upper esophageal sphincter opening. A detailed technical description of these measures and their acquisition is presented by Leonard and Kendall[13] and is only briefly summarized here. Maximum hyoid elevation is defined by measuring the distance between the hyoid position at bolus "hold" and the hyoid position at its point of maximal anterior and superior excursion during the swallow. Pharyngoesophageal sphincter opening is defined as the size of the narrowest point of opening between C3 and C6 during maximal distention for bolus passage.

DATA ANALYSIS

The data from each measured variable were aver-

TABLE 2. FINDINGS ACCORDING TO TYPE OF BOLUS

	Paste	*Liquid*	*p*
Bolus pharyngeal transit time (Pcl – B1; s)	0.91 ± 0.36	0.84 ± 0.23	.37
Oropharyngeal transit time (BV – B1; s)	0.33 ± 0.24	0.25 ± 0.13	.02
Hypopharyngeal transit time (Pcl – BV; s)	0.58 ± 0.27	0.59 ± 0.18	>.80
Onset of AE fold elevation (AEstart – B2; s)	0.27 ± 0.35	0.14 ± 0.26	.027
Time required for AE fold elevation (AEclose – AEstart; s)	0.25 ± 0.10	0.27 ± 0.15	.45
Initial hyoid movement timing (H1 – B1; s)	0.26 ± 0.33	0.22 ± 0.22	.39
Hyoid elevation versus AE fold elevation (H1 – AEstart; s)	0.02 ± 0.22	0.09 ± 0.20	.069
Time required for hyoid displacement (H2 – H1; s)	0.35 ± 0.25	0.28 ± 0.12	.051
Hyoid elevation time (H3 – H2; s)	0.17 ± 0.09	0.21 ± 0.11	.02
Distance of hyoid bone elevation (cm)	1.76 ± 0.56	1.92 ± 0.70	.17
Onset of PES opening (Pop – B1; s)	0.56 ± 0.36	0.45 ± 0.21	.03
Time of maximum PES opening (PESmax – B1; s)	0.69 ± 0.36	0.58 ± 0.23	.04
Duration of PES opening (Pcl – Pop; s)	0.33 ± 0.09	0.41 ± 0.07	<.0001
PES opening relative to bolus arrival (BP – Pop; s)	0.04 ± 0.05	0.01 ± 0.02	<.0001
Extent of PES opening (cm)	0.62 ± 0.16	0.51 ± 0.15	.0003
Maximum pharyngeal constriction (PAmax – B1; s)	0.80 ± 0.36	0.75 ± 0.24	.36

Data are mean ± SD.

AE — aryepiglottic, PES — pharyngoesophageal sphincter.

aged across all subjects according to bolus consistency. Mean values were used in *t*-test comparisons of each bolus category. A significant p value was set at .003 in order to ensure α protection by a Bonferroni correction. Interobserver measurement reliability among 4 individuals who routinely analyze videostroboscopic studies was established by 2-way analysis of variance on 15 of the normal swallow studies and was found to be greater than 90% for all of the measurements.

RESULTS

The results of the *t*-test analysis of each variable mean are reported with the p values found by the analysis.

BOLUS TRANSIT TIMING

No statistically significant difference in the timing of pharyngeal transit occurred with variations of bolus consistency. When divided into oropharyngeal and hypopharyngeal transit times, bolus pharyngeal transit did not vary with bolus type (Table 2). An increase in the speed of oropharyngeal transit for a liquid bolus was identified, but was not statistically significant.

SWALLOW GESTURE TIMING AND DISTANCE

Aryepiglottic Folds. The onset of aryepiglottic (AE) fold closure did not differ between the two bolus categories. Along with the increase in the speed of bolus oropharyngeal transit, AE fold elevation appeared to occur earlier with a liquid bolus, but this was not statistically significant. Once the AE folds began to elevate, there was no difference in the time required for arytenoid elevation with variations of bolus consistency (Table 2).

Hyoid Bone. The onset of hyoid bone elevation relative to the start of the swallow did not vary with changes in bolus consistency. When the onset of hyoid bone elevation was directly compared with the onset of AE fold elevation, the difference between liquid and paste bolus categories was not significant. The time required for the hyoid bone to reach maximal elevation was not different when a liquid bolus was compared to a paste bolus. The duration of hyoid bone elevation was the same for both bolus categories (Table 2).

An adequate extent of hyoid elevation is considered to be required for adequate opening of the upper esophageal sphincter.[12,14-16] The distance of hyoid bone elevation was not different between the two bolus groups (Table 2).

Pharyngoesophageal Sphincter. The onset of PES opening and the time at which the PES reached maximum distention occurred earlier for a liquid bolus than for a paste bolus, but the difference was not statistically significant. The duration of PES opening was significantly longer for a liquid bolus than for a paste bolus. The opening of the PES relative to the bolus arrival also was different between bolus categories. The opening of the PES relative to the arrival of the bolus at the sphincter (BP) was much earlier for a paste bolus, perhaps demonstrating the need to generate negative pressures to drive the higher-viscosity bolus inferiorly (Table 2).

The PES must open adequately to allow bolus passage into the esophagus. The extent of PES opening was significantly larger for the paste bolus category

(Table 2).

Pharynx. There was no difference detected between bolus categories in the timing of maximum pharyngeal constriction (Table 2).

SUMMARY

We detected no difference in the duration of the overall bolus pharyngeal transit time (as defined by the interval between the moment the head of the bolus passes the anterior nasal spine and the moment the tail of the bolus passes through the PES). The oropharyngeal bolus transit trended toward being faster with a liquid bolus. Variation of gestures in response to changes in bolus consistency included a trend toward earlier elevation of the AE folds with a liquid bolus, likely in response to the earlier arrival of the bolus at their level. Although the onset of hyoid bone elevation and the time required for elevation were the same for both bolus consistencies (apparently unrelated to the location of the bolus in the pharynx), the duration of hyoid bone maximal elevation trended toward being longer for liquid boluses. The extent of hyoid elevation was not affected by changes in bolus viscosity. Finally, the upper esophageal sphincter demonstrated a trend toward opening earlier relative to the onset of the swallow for liquid boluses (but significantly closer in time to the arrival of the bolus at the sphincter than for paste boluses). The PES also trended toward reaching maximal distention earlier and stayed open significantly longer during the swallow of a liquid bolus (Table 2). The maximum size of PES opening was significantly larger during the swallow of a paste bolus (Table 2).

DISCUSSION

Although differences in measurement methods exist, this study supports the findings of previous scintigraphic studies that show no difference in bolus pharyngeal transit duration with variations in bolus consistency. Normal individuals probably respond to variability in the viscosity of the bolus so that the time of bolus transit is maintained as constant.

Despite the findings of previous studies that demonstrate an increased oral and tongue muscular contraction generated during propulsion of a paste bolus from the oral cavity into the pharynx, this study demonstrated a trend wherein the flow of a paste bolus was not as rapid as that of a liquid bolus through the first part of the pharyngeal phase of swallowing.[1,6,7] It seems likely that the flow characteristics of the liquid bolus enable it to move more rapidly despite the application of a lesser muscular driving force. In addition, the need for earlier airway protection resulting from the earlier arrival of the bolus at the level of the glottis prompts a trend toward earlier elevation of the AE folds toward supraglottic closure.

Hyoid bone elevation time appeared to correlate with the duration of PES opening, both of which were prolonged for the liquid bolus. The distance of hyoid bone elevation was not affected by bolus category, and the larger opening size of the PES during a paste swallow may have been in response to intrabolus pressure, rather than due to external traction on the sphincter by hyoid-larynx elevation. Not surprisingly, the paste bolus appeared to "hold together" in a ball more than the liquid bolus and passed through the PES more rapidly, but it required a larger opening in order to pass.

In terms of PES functioning, the findings of this study are in contradiction to the findings of Dantas et al,[1] who found that the PES remained open longer with more viscous bolus consistencies even at small bolus volumes, such as were investigated in this study. In contrast, a highly significant prolongation of PES opening was demonstrated with a liquid bolus in our study. Perhaps differences in defining the location of the PES or differences in subject numbers can account for this reversal. This study evaluated bolus viscosity accommodation in 60 subjects, as opposed to the 10 subjects evaluated by Dantas et al.

Surprisingly, differences in pharyngeal constriction in terms of timing were not identified. This study did not evaluate the force of muscular contraction, and because the pharynx is routinely almost completely obliterated on both paste and liquid boluses, it is possible that differences could not be identified by videofluoroscopy. This is one of the shortcomings of this particular measurement technique. We generally believe that patients found to have complete pharyngeal obliteration on videofluoroscopy demonstrate adequate muscular contraction. It must be kept in mind, however, that the definitive evaluation of pharyngeal pressures developed by muscular contraction requires manometry.

This study evaluated a small bolus size and demonstrates minimal findings in terms of variation in swallow gestures that can be interpreted as adaptations for the management of a more viscous bolus. In fact, the small paste bolus appears to almost require *less* effort to swallow in terms of early airway protection. This finding supports therapeutic strategies for management of dysphagia that include the substitution of thicker consistencies and the avoidance of thin liquids in patients who have difficulties with airway protection. In general, changes identified by videofluoroscopy appear to be related to the duration of hyoid elevation coupled to PES opening. Bolus flow properties and pressures likely also influence the findings, including oropharyngeal transit duration and the size of PES opening. Further studies are required to understand the mechanisms, both central and peripheral, that control the observed adaptations.

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RELATIONSHIP BETWEEN TRANSGLOTTAL PRESSURE AND FUNDAMENTAL FREQUENCY OF PHONATION, WITH EFFECTS OF DEHYDRATION PRODUCED BY ATROPINE, IN HEALTHY VOLUNTEERS

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We investigated the effects of the intravenous injection of atropine sulfate on the relationship between the fundamental frequency of phonation (F0) and the magnitude of F0 change per unit change in transglottal pressure (dF/dP) in 4 healthy male volunteers. Before the injection of atropine, we observed a nonlinear or V-shaped relationship between dF/dP and F0. After the injection, the values for dF/dP were reduced at a lower F0, whereas the values were unchanged at a higher F0. The results support our hypothesis that the relationship between dF/dP and F0 reflects adjustments in the length and depth of the vocal folds in controlling F0.

KEY WORDS — fundamental frequency, phonation, transglottal pressure, vibration, vocal fold.

INTRODUCTION

We previously described the relationship between transglottal pressure and the fundamental frequency of phonation (F0) in normal subjects.[1,2] The change in F0 per unit change in transglottal pressure (dF/dP) shows a nonlinear or V-shaped relation to F0. When F0 is increased from low to high within a modal register, the values for dF/dP fall until F0 reaches values of 200 to 250 Hz. The values for dF/dP increase when these frequencies are exceeded.

On the basis of results of experiments conducted in dogs, as well as of theoretical analysis, Titze[3] reported that the relationship between dF/dP and F0 is determined by the amount of elongation of the vocal folds, the dF/dP being smaller in elongated vocal folds, at least within the modal register. An instantaneous extension of the vocal folds within each cycle of vibration (the so-called dynamic strain) was held responsible. These results coincide well with those of previous studies in humans that found smaller values for dF/dP at a higher F0 within the modal register.[4,5]

In humans, the F0 is regulated not only by the elongation of the vocal folds, but also by the restriction of the vibrating depth of the vocal folds.[6-9] The effect of vibrating depth on the values of dF/dP has also been described by Titze.[3] That author anticipated that values of dF/dP would increase when the vibration is restricted to the edge of the vocal fold, although he stated that further investigations would be required.

On the basis of the above studies, we expected that elongation would reduce the values for dF/dP and that restriction of the vibrating depth would increase them. Because the elongation and the restriction work simultaneously to raise the F0, the integrated results of these two actions would determine the relationship of dF/dP to F0. We observed in humans a V-shaped relationship between dF/dP and F0.[2] Concerning the underlying mechanism, we hypothesize that an elongation prevails in determining the dF/dP at a lower F0, whereas with a higher F0, the restriction in depth of vibration prevails.

Atropine sulfate induces dehydration of the vocal folds by reducing the secretory function of the laryngeal glands. When the vocal folds are dehydrated, their internal friction should increase,[10] and thus, their strain (elongation per unit length of the vocal fold) would be reduced. We thought that the dynamic strain described by Titze[3] could be affected by dehydration of the vocal folds, altering the relationship between dF/dP and F0.

Several reports have described the effects of dehydration on the vibration of the vocal folds. In canine experiments, Finkelhor et al[11] found that dehydration increased the phonation threshold pressure (PTP). Verdolini-Marston et al[12,13] measured PTP in humans and observed that it increased during dehydration, with the increase being the greatest during high-pitched phonation (lowest note plus 80% of the total pitch range). The increased friction[13] of the vocal folds requires a larger subglottal pressure to initiate vibra-

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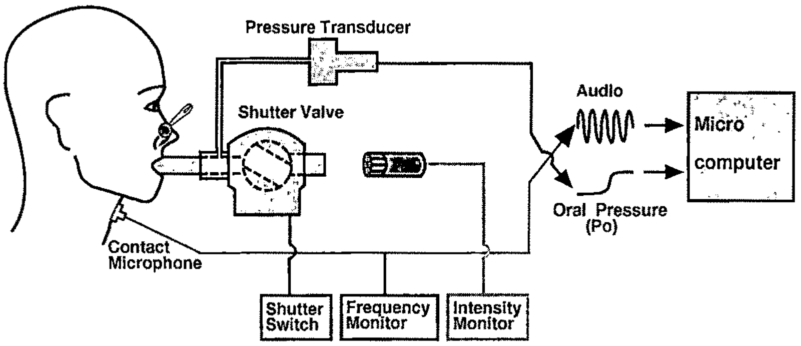

Fig 1. Diagram of system used to evaluate relationship between fundamental frequency of phonation (Fo) and magnitude of Fo change per unit change in transglottal pressure (dF/dP).

tion, since it deprives the folds of the energy required to produce vibration. It is expected that dehydration of the vocal folds would also affect the amount of dynamic strain during sustained phonation, and thus, the values of dF/dP.

Our present objectives were to determine 1) whether the intravenous injection of atropine would alter the relationship between dF/dP and F0 in humans and 2) whether the results would be reasonably explained by our assumption regarding the mechanism underlying the V-shaped relationship between dF/dP and F0. As was described earlier, we hypothesize that an elongation prevails in determining the dF/dP at a lower F0, whereas at a higher F0, a restriction in depth prevails.

SUBJECTS AND METHODS

Subjects. Four healthy adult Japanese men, ages 26, 27, 29, and 35 years, volunteered for the study. None had a history of vocal or singing training, had any problem with phonation or articulation, or had any lower respiratory tract disorder or heart disease. Informed consent was obtained from each subject. Each was well informed about the objective of the study, the possible anticholinergic effects of atropine on the body, and his safety and well-being as related to atropine administration. The general condition of each subject was monitored throughout testing by an anesthesiologist who was equipped with an emergency kit to treat any untoward effects of atropine as might be required.

Equipment. A change in transglottal pressure was to be produced by a sudden increase in the intraoral pressure during sustained phonation. As illustrated in Fig 1, a shutter valve mounted on the mouthpiece interrupted airflow through it, thus increasing the intraoral pressure. This valve (Nagashima Air Flow Interrupter NK 100, Tokyo, Japan) consists of a rotating cylinder with a hole in it to allow the passage of air. The cylinder was programed to rotate 45° by a step motor, reducing by one seventh the diameter of the airway. The cylinder remained in that position for 300 ms and was then re-rotated to the original position; the time required to rotate the cylinder was 25 ms. Intraoral pressure (Po; in centimeters of water) was sensed through a metal tube (inner diameter, 2.0 mm) that was connected to a strain-gauge transducer (Biophysiograph 180 System, San-ei, Tokyo). The voice signal was picked up by a contact microphone (SH-01, Rion Co, Ltd, Tokyo) positioned on the anterior portion of the subject's neck. Voice and pressure signals were recorded on a data recorder (TEAC

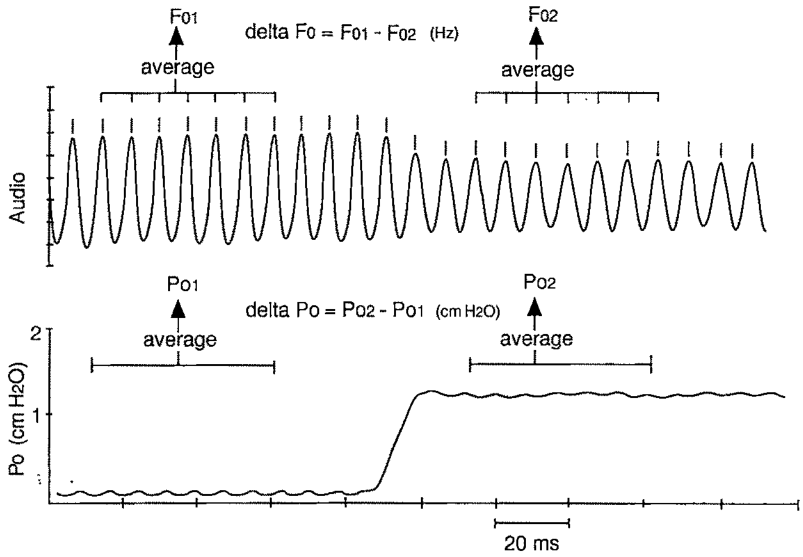

Fig 2. Examples of simultaneous recordings of voice (upper wave) and intraoral pressure (Po; lower wave).

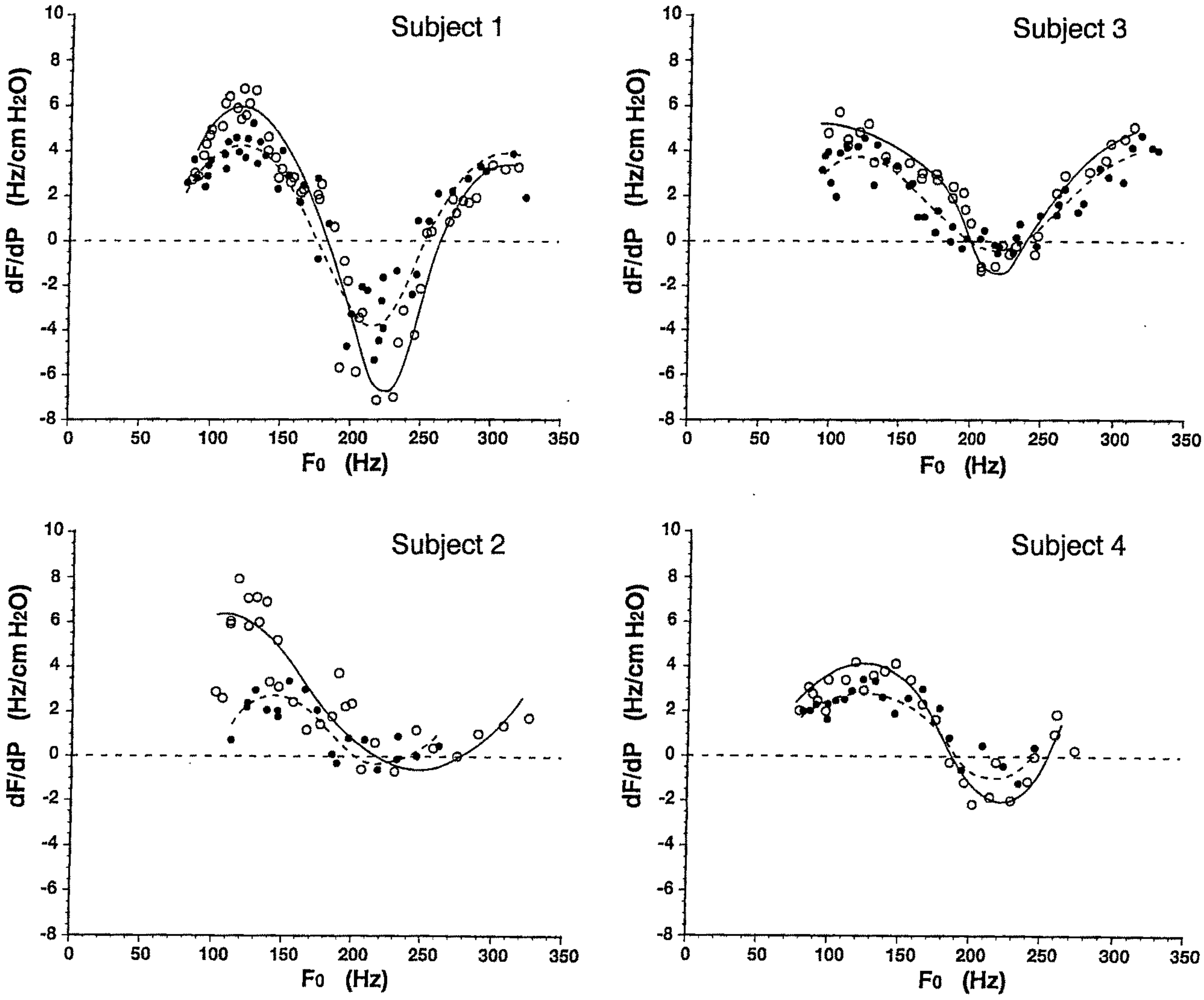

Fig 3. Relationship between dF/dP and Fo in 4 healthy men. Open circles represent values obtained before injection of atropine, and closed circles represent those obtained after injection. General trend of relationship between dF/dP and Fo in each subject before injection is shown as continuous curve, and interrupted curve indicates results obtained after injection. Relationship shows quasi–V-shaped pattern in all 4 subjects before and after injection.

MR-10, Tokyo) for analysis by a computer.

Procedures. Each subject was evaluated in a sitting position. An airtight seal was maintained by placing the lips tightly around the mouthpiece. Nose clips were also applied to prevent the escape of air through the nostrils. Each subject was instructed to take several breaths before each phonation, and then to utter the vowel /o/ at a constant pitch and loudness in a single breath. Vocal intensity was based on the individual's comfort level. The pitch, which varied as described below, was determined to the nearest note on a piano. After we confirmed that the intensity and pitch had reached a stable level, we pressed the shutter valve switch. As long as this switch was pressed, the valve was closed and re-opened each second. In this study, the valve closed 5 times during most trials. After the pitch was raised by 1 note, the procedure was repeated. We failed to obtain 5 closures at all higher pitches, because the subject could not maintain a prolonged and stable phonation. The pitch was increased until at least 3 closures were successively obtained at each pitch within the modal register, as determined subjectively by the examiner. If the examiner had difficulty in making a decision about the trial success, that particular trial was discarded. The magnitude of change in transglottal pressure that was produced by the valve was dependent on the Fo and the intensity of each trial. The subject was instructed not to utter a breathy or pressed phonation, as detected by the examiner. We did not regulate the intensity of phonation, which usually increased with a higher Fo. From a published report, the intensity seems to have little influence on the values for dF/dP.[14] In each trial, the stability of the Fo and the intensity were monitored on the oscilloscope of the Phonolaryngo-

DIFFERENCES BETWEEN PRE-ATROPINE AND POST-ATROPINE dF/dP

Subject	Fo (Hz)	Mean dF/dP After Atropine	Mean dF/dP Before Atropine	Difference of Means	Wilcoxon t-Test t	Wilcoxon t-Test p
1	<100	2.429	3.363	−0.934	−2.470	.0217*
	−140	4.109	5.170	−1.061	−3.320	.0023*
	−180	2.937	2.594	0.343	0.947	.3561
	−220	−1.925	−3.080	1.155	0.872	.3968
	−260	−0.931	−2.886	1.955	1.744	.1016
	≥260	3.062	2.149	0.913	1.873	.0784
2	<140	1.932	5.423	−3.491	−3.839	.0020*
	−180	2.396	2.454	−0.058	−0.077	.9405
	−220	0.202	1.657	−1.455	−2.435	.0351*
	≥220	0.315	0.413	−0.098	−0.204	.8466
3	<140	3.548	4.644	−1.096	−2.918	.0096*
	−180	1.817	2.965	−1.148	−2.421	.0360*
	−220	0.006	0.641	−0.636	−1.116	.2833
	−260	0.418	0.202	0.217	0.432	.6751
	≥260	2.990	3.603	−0.612	−0.930	.3696
4	<100	2.110	2.537	−0.427	−1.524	.1585
	−140	2.947	3.484	−0.537	−2.181	.0570
	−180	2.607	2.860	−0.253	−0.345	.7441
	−220	0.440	−0.930	1.370	2.426	.0596
	≥220					

dF/dP — magnitude of change in Fo per unit change in transglottal pressure; Fo — fundamental frequency of phonation.
*$p < .05$.

gram (SH-01, Rion Co). After each subject had completed the above procedures at baseline, he received an intravenous injection of atropine sulfate 0.5 mg. Ten minutes later, the procedure was repeated to evaluate the effects of atropine on the values for dF/dP. The "no treatment–treatment" sequence was the same in each of the 4 subjects. The amount of hydration at baseline was not controlled, and it varied according to subject. The amount of dehydration induced by atropine also varied according to subject. Since our objective was to evaluate the qualitative effects of atropine in each individual, we did not think that an order effect would invalidate our findings.

Calculation of dF/dP. The voice signal and Po were recorded on a data recorder and were analyzed by a microcomputer (Signal Processor 7T18, San-ei). A typical computer display is shown in Fig 2. The upper wave of the display is the voice signal, and the lower wave is the Po. The sampling frequency was 20 kHz. Among the 5 trials of valve closure, which sometimes involved 4 or 3 closures, depending on the subject, the trial that showed the most stable voice signal on the display was selected for analysis.

The following parameters were evaluated: F01 (F0 before the increase in Po); F02 (F0 after the increase in Po); delta F0 (F01 – F02); Po1 (Po corresponding to F01); Po2 (Po corresponding to F02); delta Po (Po2 – Po1); and dF/dP (delta F0/delta Po). F01 was determined by averaging the peak intervals of the F0 cycles during the 50 ms just before the pressure buildup. Similarly, F02 was determined for a 50-ms window just after the pressure buildup. A 50-ms interval was used to minimize the effects of the involuntary response of the laryngeal muscles to the sudden change in pressure.[4,14,15] Thus, the number of peak intervals contained within the 50 ms differed, depending on the F0 itself. At 100 Hz, for example, 4 peak intervals were averaged. Po1 and Po2 were obtained by averaging the Po values during the same time intervals (50 ms) used to determine the F01 and F02, respectively.

RESULTS

The dF/dP was plotted against the F0 for the 4 subjects as shown in Fig 3. The relationship showed a quasi–V-shaped pattern for all 4 subjects before and after the intravenous administration of atropine. The Table shows the differences between pre-atropine dF/dP and post-atropine dF/dP for each F0 range, with *t*-values and probability (p) obtained by the Wilcoxon *t*-test. The post-atropine difference was evaluated at each range of F0 spanning 40 Hz. The 40-Hz range was used to obtain enough samples for statistical analysis. Below 140 Hz, the values for dF/dP were smaller after the injection of atropine in subjects 1, 2, and 3. Above 220 Hz, there was no post-atropine difference in any subject. Overall, atropine reduced the values in the lower region of F0, but not in the higher region.

All 4 subjects reported a dry throat after administration of atropine. No hoarseness was perceived by either the subjects or the examiners; however, no objective tests of hoarseness were performed.

DISCUSSION

The intravenous administration of atropine induces bronchodilation and reduces airway resistance,[16] and it inhibits secretory function. A change in resistance would affect the absolute value of the transglottal pressure induced by the shutter valve in our system. However, because we analyzed the relative extent of the F0 change, changes in resistance did not seem to affect our results.

The dF/dP is determined by dynamic strain, that is, the instantaneous stretch of the vocal folds during each cycle of vibration. The length of the vocal folds and the depth of vibration are considered to be parameters for dF/dP.[3] The values for dF/dP decrease when the vocal folds are increased in length or stretched, and increase when the depth of vibration is reduced. The injection of atropine causes dehydration, and thus might increase the internal friction of the vocal folds during vibration.[10] The dynamic strain would be decreased because of the increase in internal friction. Thus, we expected the dF/dP to be smaller after the injection of atropine. This expectation was met at a low F0 (below 140 Hz), but not at a high F0 (above 140 Hz). The vocal folds are fully stretched at around 140 Hz. Thus, the internal friction of the vocal folds should have been quite high, such that atropine would produce no obvious effect on the vocal folds (ie, a ceiling effect). Thus, the mechanism that determines dF/dP at a low F0 would seem to differ from that which determines dF/dP at a high F0; this finding does not contradict our hypothesis regarding the dF/dP-F0 relationship.

Concerning the negative values of dF/dP, Isshiki[5] reported that in an experiment in dogs, in a particular region of the F0, a sudden increase in the vibrating portion of the vocal folds was observed when the transglottal pressure was suddenly increased during sustained phonation, leading to an F0 decrease despite an increase in transglottal pressure. We speculate that a sudden reduction in the depth of vibration occurs after valve closure, which leads to an increase in F0 despite a reduction in transglottal pressure. Although our method of altering the transglottal pressure differs from that of Isshiki, the dF/dP would show negative values in both cases. Atropine had no effect on the negative values of dF/dP.

Our hypothesis concerning the mechanism of the dF/dP-F0 relationship is, as we described above, that a change in vocal fold length prevails in the determination of the dF/dP at a lower F0, whereas a change in the depth of vocal fold vibration prevails at a higher F0. Previous studies[6,17,18] indicate that the relationship between the length of the vocal folds and the F0 reaches a plateau at a higher F0, even within the modal register. A comparison of these previous studies with our present results allows us to expand our hypothesis. That is, at a higher F0, F0 would be controlled by a reduction in the depth of vibration rather than by a change in the length of the vocal folds. So, the relationship between dF/dP and F0 would reflect length-versus-depth adjustments for F0 control. On the left half of the (on a graph) V-shaped relationship between dF/dP and F0, the change in length, and on the right half of the V, the change in depth, would prevail in regulating F0. This hypothesis is interesting from a physiological point of view. The V-shaped relationship suggests that even within the modal register, there might be a change in the manner of raising the F0 (elongation versus depth of the vocal folds). From a clinical viewpoint, it would be interesting to investigate whether pathological changes in the vocal folds would alter the manner in which the F0 is controlled. We anticipate that the V-shape would shift to the right or to the left to cope with the pathological change in the vocal folds.

CONCLUSION

The effects of atropine on the relationship between dF/dP and F0 were investigated in 4 healthy men. The values of dF/dP were reduced at a lower F0, but not at a higher F0. The present observations and those of previous studies regarding the relationship between vocal fold length and F0 suggest that the relationship between dF/dP and F0 reflects length-versus-depth adjustments for F0 control. On the left half of the (on a graph) V-shaped relationship, a change in length, and on the right half of the V, a change in depth, would be responsible for regulation of F0.

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VOCAL FOLD PARESIS OF CHARCOT-MARIE-TOOTH DISEASE

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No cohesive overview of vocal fold abnormalities associated with Charcot-Marie-Tooth disease (CMT) has been presented in the literature. This study examines a patient in depth and compares the findings with those of published reports to characterize the features of vocal fold paresis in CMT. The affected patient was investigated with nerve conduction testing, laryngeal electromyography, endoscopy, and laryngeal sensory testing. Ten published cases were reviewed for similarities and differences. Vocal fold paresis has been observed in 11 CMT patients ranging in age from 8 to 80 years. Two cases have occurred in the context of CMT type 1, and 9 in CMT type 2. Seven of the 11 cases (64%) were clearly bilateral; only 2 of the 7 cases (29%) required tracheotomy, and both were in children. The electromyographic findings were typical of reinnervation. Sensory findings were present, but did not represent significant disability in the 1 patient so studied. We conclude that CMT does not spare the cranial nerves, as has been previously thought. Furthermore, vocal fold paresis is not restricted to CMT type 2 and should not be considered a hallmark of that category. The available evidence suggests that the neural deficit evolves gradually, may exhibit partial recovery, and often escapes notice for a time. Vocal fold abnormalities are most often bilateral. Because the deficit is generally well tolerated in adults, many cases have probably been overlooked, and no conclusion regarding incidence is possible. Nevertheless, the potential for airway compromise exists, especially in children. Respiratory complaints of CMT patients should be thoroughly investigated.

KEY WORDS — Charcot-Marie-Tooth disease, laryngeal electromyography, vocal fold paralysis.

Charcot-Marie-Tooth disease (CMT), also known as hereditary motor and sensory neuropathy or peroneal muscular atrophy, is a genetic disorder with heterogeneous clinical features that has weakness and sensory loss in the distal limbs as its most prominent features. It accounts for 80% to 90% of all genetic neuropathies, which in turn make up about 20% of all neuropathies. In the United States, this amounts to an incidence of 42 per 100,000.[1] It is more prevalent than myasthenia gravis.

Electrophysiological studies, and more recently genetic analysis, have yielded an increasingly sophisticated classification scheme. Currently, CMT is divided into 4 major types, each with several subtypes, as well as an X-linked form. Even though all have essentially the same phenotypic presentation, variability in clinical features persists within each class. Vocal fold and respiratory muscle abnormalities have been reported in association with CMT type 2C, an axonal type.[2,3]

We present a case of bilateral vocal fold paresis in a patient with the demyelinating form of CMT, designated CMT1. We further present laryngeal electromyographic findings and sensory testing for the first time in such a case. Review of published reports reveals that vocal fold abnormalities are not unique to CMT2C, although laryngeal findings and clinical features appear remarkably similar in most patients. This study undertakes to characterize the vocal fold findings typical of CMT.

CASE STUDY

A 34-year-old man complained of intermittent hoarseness over the past year. This complaint was associated with prolonged voice use, and manifested only in the Southeast Asian city in which he lived and worked. He was free of any voice symptoms during his visits to the United States. He had never had voice symptoms until the past year, and he denied any dyspnea.

At the age of 11 years, he came to the attention of physicians because of lower-extremity weakness. At that time, he underwent electromyography and nerve conduction studies and muscle and nerve biopsies that established a diagnosis of CMT1. An extensive family history was taken. The patient's siblings and father were also examined, and the father underwent nerve conduction testing. There was no sign of this disorder in any other family member. Since diagnosis, the patient's disease had progressed slowly.

Marked inspiratory stridor during speech was immediately evident in this patient, although he did not

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TABLE 1. LARYNGEAL ELECTROMYOGRAPHIC FINDINGS IN REPORTED CASE

Muscle	*Spontaneous Activity*	*Voluntary Activity*	*Synkinesis*	*Maximum Amplitude (mV)*
Right cricothyroid	None	Numerous giant waves and some polyphasic potentials, some with bizarre morphology	Not tested	9.8
Left cricothyroid	None	Numerous giant waves with picket-fence interference pattern; some polyphasic potentials	Not tested	4.6
Right thyroarytenoid	None	Frequent giant waves and polyphasic potentials	Marked inappropriate activity on inspiration	0.7
Left thyroarytenoid	None	Some giant waves and polyphasic potentials	Not tested	2.0
Right posterior cricoarytenoid	None	Diminished electrical activity; only rare polyphasic potentials	Minimal	0.5

complain of it. He admitted that other physicians had remarked on this finding on previous occasions, but he himself remained unaware of any noisy breathing. Upon questioning, he reported rare episodes of dysphagia with choking.

Examination revealed many of the stigmata of CMT, including distal atrophy and weakness, high arches of the feet, absent deep tendon reflexes, and sensory loss in a stocking-glove distribution. The peripheral nerve diameter was enlarged to palpation.

On electrophysiological testing, the motor nerve conduction studies demonstrated marked prolongation of distal latency, low amplitude, and slow conduction velocity (25 to 31 m/s), affecting all nerves tested to a congruous degree. Sensory nerve responses were not obtainable. The facial motor study showed a greater than 40% increase in latency and muscle action potential (MAP). Bilateral phrenic nerve studies showed a comparable increase in latency (2 times normal) with low-amplitude MAPs. Concentric-needle electromyography demonstrated evidence of mild spontaneous activity (fibrillations) in distal lower-extremity muscles and evidence of motor units of increased amplitude, duration, and polyphasia with a discrete recruitment pattern in the proximal and distal lower-extremity muscles, consistent with a chronic neurogenic process. In summary, these electrophysiological findings are consistent with a hereditary demyelinating polyneuropathy.

The diagnosis was further supported by a reexamination of nerve and muscle sections obtained during the initial investigations of 1977. The nerve biopsy demonstrated moderate to severe loss of myelinated fibers. All of the identifiable myelinated fibers were small and had abnormally thin myelin sheaths. Most, if not all, of these thinly myelinated fibers were part of an onion bulb. Sparse, concentrically oriented nuclei, presumably those of Schwann cells, surrounded these fibers in transverse sections. The muscle biopsy findings were consistent with moderate neurogenic atrophy.

A head and neck examination was remarkable for a mild facial droop on the right, although there was no frank paralysis. Extraocular movements, facial sensation, lingual and palatal motion, and trapezius and sternocleidomastoid function were all intact and symmetric.

Stroboscopic evaluation revealed complete vocal fold immobility on the left and nearly complete immobility on the right. Maximal abduction resulted in a 2- to 3-mm glottic aperture. Adduction resulted in a height mismatch, with the left vocal fold coming to midline slightly above the right. Nevertheless, the voice quality was good. There was no evidence of tremor. Pulmonary function testing yielded a flow-volume loop consistent with variable extrathoracic obstruction, although there was also a reduced forced vital capacity (4.00 L; 74% of predicted). Laryngeal electromyography was performed with a monopolar needle electrode as previously described by the authors.[4] The findings are summarized in Table 1. There was no evidence of spontaneous activity in any of the muscle tested. The thyroarytenoid and cricothyroid muscles on both sides showed giant waves and polyphasic potentials, suggestive of reinnervated units. The right thyroarytenoid muscle demonstrated inappropriate activity during inspiration, suggesting synkinetic reinnervation. The posterior cricoarytenoid muscle was electrically quiet. Laryngeal psychophysical sensory testing was administered according to a method described elsewhere.[5] The right-sided threshold was 4.46 ± 0.41 mm Hg air pulse pressure (APP; mean ± SD); and the left was 2.78 ± 0.44 mm Hg APP (normal, <4.0 mm Hg APP).

Because the patient never experienced hoarseness during the period of his evaluation, its cause remains a matter of conjecture. It is not the direct result of his vocal fold paresis, as his speech remained unimpaired throughout his stay in the United States. After extensive discussion regarding his bilateral paresis and the possibility of life-threatening respiratory embarrassment, he declined intervention.

DISCUSSION

Charcot-Marie-Tooth disease was initially described by the eponymous authors in 1886 on the basis of distal muscular atrophy that they recognized was familial.[6,7] In fact, most forms of CMT are inherited in an autosomal dominant manner. Although its cause was obscure, Tooth suggested neuropathy. Electrophysiological studies performed in the 1970s and 1980s confirmed that CMT was primarily a neuropathy, and 2 forms were identified. The demyelinating form features markedly slowed nerve conduction velocity (NCV), but normal or borderline-reduced compound muscle action potentials (CMAPs) and sensory nerve action potentials (SNAPs), and is now known as CMT1. The axonal type demonstrates normal or slightly reduced NCV, but low-amplitude CMAPs and SNAPs, and is known as CMT2, or neuronal CMT. Molecular genetic studies have established subclasses of CMT1, as well as CMT3, a severe, early-onset demyelinating form; CMT4, an autosomal recessive form; and CMTXA, an X-linked dominant variety. Type 2 CMT has been divided into at least 4 subgroups by phenotypes and genetic linkage studies, without confirmed gene isolation. In addition, genetic similarities have placed hereditary neuropathy with liability to pressure palsies, which is usually a recurrent peripheral mononeuropathy, in the group of CMT disorders. Excellent reviews of the nosology of CMT are available.[1,8]

In the majority of cases, the disease begins to manifest in the first or second decade of life and progresses slowly. In general, life expectancy is not markedly diminished, with the important exception of patients with CMT2C. This is attributed to aspiration and respiratory complications from laryngeal abnormalities,[2,8] discussed further below.

Characteristic clinical features include distal muscle weakness and atrophy of the legs with bone changes over the long term, resulting in pes cavus, hammer toe, and talipes equina varus. "Stork leg" or "inverted champagne bottle thigh" describes the appearance of the lower extremity with atrophied muscles. Upper-extremity findings are similar, and patients may have claw hand. Sensory loss is often subtle, and most often affects vibration. A useful clinical distinction between forms is the palpable enlargement of peripheral nerves in CMT1 and CMT3, present in about half of patients.[8] An affected greater auricular nerve is particularly helpful to the examining otolaryngologist. Also of note for the otolaryngologist, bilateral, progressive, mild to severe sensorineural hearing loss has been documented in patients with CMT1, CMT2, CMT4, and CMTXA.[9] Audiological findings can be heterogeneous, even within subtypes of CMT.

Laryngeal abnormalities in CMT were originally reported by Holinger et al,[10] culled from their huge series of 389 cases of bilateral vocal fold paralysis. They described a 10-year-old boy who required tracheotomy because of acute respiratory distress, and his 48-year-old mother, who, although she had stridor, was not inconvenienced by it. Both demonstrated bilateral vocal fold immobility on indirect laryngoscopy. No other cranial nerve deficits were found. Holinger et al were able to follow the boy over a 7-year period, during which his laryngeal examination findings did not change. He was hospitalized on 4 occasions for respiratory compromise, and required tracheotomy a second time 5 years after the first. However, he was decannulated successfully without other procedures on both occasions. On the basis of clinical examination findings demonstrating the CMT phenotype and the results of NCV studies, which were normal in the boy and inconsistently prolonged in his mother, these cases were tentatively designated CMT2. Electromyography confirmed the chronic axonal degeneration consistent with CMT2. Further classification was not possible at the time of the report.

Fourteen years later, an isolated case of bilateral vocal cord paralysis was reported in a 42-year-old man with demyelinating neuropathy, presumed CMT1, diagnosed by sural nerve biopsy and nerve conduction study.[11] This patient presented in acute distress. In addition to laryngeal findings, there was severe bilateral sensorineural hearing loss with an undetectable auditory brain stem response, mild bilateral weakness of the facial muscles, and diaphragm weakness.

In another report, diaphragm findings were noted in conjunction with vocal fold abnormalities in 2 kinships.[2] Unfortunately, the larynx was not examined in all 8 patients of the first kinship with respiratory or voice complaints, and when it was, the abnormalities were not described individually. Thus, "paresis of one or both vocal cords was found in 3 of the 4 patients" and "in the fourth, mild paresis was inferred from the exhibited flaccid dysphonia."[2(p609)] There is also the suggestion that laryngeal electromyography was performed on 1 patient, but results are not presented. Of interest, phrenic nerve stimulation was evaluated in all 8 patients, and the CMAPs were found to be decreased or absent in 6.

The propositus of the second kinship was a 32-

TABLE 2. SUMMARY OF ALL REPORTED CASES OF VOCAL FOLD PARESIS IN CMT

Case	*Authors*	*Age (y)*	*Sex*	*CMT Type*	*Laryngeal Findings*	*Age of Onset of Complaints*	*Comments*
1	Holinger et al[10]	10	M	2?	Bilateral VF paresis	10 y	Acute distress; tracheotomy ×2; decannulated
2	Holinger et al[10]	48	F	2?	Bilateral VF paresis	Unknown	No acute distress, but stridulous, mother of patient in case 1
3	Fukuda et al[11]	42	M	1	Bilateral VF paresis	Unknown	Acute distress; CN VII and VIII dysfunction; diaphragm weakness
4	Dyck et al[2]	8	F	2C	Bilateral VF paresis	8 mo	"Asthma"; paresis found incidentally
5	Dyck et al[2]	32	F	2C	R VF paralysis; L VF weakness	6 mo	Permanent tracheostomy; mother of patient in case 4
6-9	Dyck et al[2]	17-80	M and F	2C	"Mild paresis," unilateral VF paresis, or bilateral VF weakness	1st to 6th decades	Cases from same kinship, not described individually
10	Dray et al[3]	39	F	2	Incomplete glottic closure	38 y	EMG evidence of LMN lesion of CN X
11	This report	34	M	1	Bilateral VF paresis	Unknown	No acute distress, but stridulous

CMT — Charcot-Marie-Tooth disease, VF — vocal fold, CN — cranial nerve, EMG — electromyographic, LMN — lower motor neuron.

year-old woman who had episodes of stridor beginning at 6 months of age, culminating in tracheotomy at 3 years of age for "partial vocal cord paresis"[2(p613)] that evolved into an immobile right vocal fold and a minimally mobile left fold 3 years later. She retained her tracheotomy as an adult, although no mention was made of laryngeal examination subsequent to age 6. The status of the diaphragm was not mentioned. Her daughter had had respiratory difficulties beginning at 8 months of age, and carried the diagnosis of asthma until age 6, when bilateral vocal fold paralysis was diagnosed at intubation for an unrelated surgical procedure. She did not require a surgical airway.

These affected members of these 2 kinships all demonstrated the normal NCV and reduced CMAPs of CMT2. However, genetic studies failed to discover abnormalities at either of the 2 chromosome loci for CMT2A or CMT2B. Therefore, their disease was classified as CMT2C. The description of these 2 kinships has established vocal fold abnormality and diaphragm paresis as hallmarks of CMT2C.

Recently, Dray et al[3] reported laryngeal electromyographic findings in a patient with CMT2 (on the basis of normal NCV), but with intact vocal fold motion. They identified evidence of a lower motor neuron lesion of the vagus nerve. No mention was made of genetic studies, but the case was designated as CMT2C on the basis of the presence of laryngeal abnormalities.

The cases discussed above, along with the case presented in this paper, are summarized in Table 2.[2,3,10,11]

Plainly, the pathological process of CMT can affect the vagus nerve. Cranial nerves are not spared, as was stated in some older descriptions. However, it is notable that the cranial nerve in question (vagus) travels the longest course of the 12, and because of its length, it has the same vulnerability as the nerves innervating the distal musculature of the extremities.

Moreover, bilateral involvement is the rule, present in at least 7 of the 11 cases of vocal fold paresis discussed. No more than 2 patients had unilateral vocal fold immobility. Conclusions regarding these cases are difficult to draw, since they are not presented in detail. Symmetric findings are prominent features of hereditary neuropathy.[1] In this respect, the laryngeal abnormalities are consistent with the other clinical findings of CMT.

We are struck by how well CMT patients, including our own, tolerate bilateral vocal fold paresis, a situation that often results in rapid and sometimes dramatic respiratory decompensation. Only 2 of the 7 patients with bilateral paresis required tracheotomy, and 1 of these was decannulated without additional procedures. At least 2 additional patients were stridulous, but accepted this as a matter of course. The patient presented in this paper insisted that he was unaware of any noise associated with his breathing. This is probably the result of insidious onset that allows some reinnervation and recovery of function. Our own electromyographic findings and those of Dray et al[3] show evidence of reinnervation, and we demonstrate synkinetic patterns of muscular activity as well. The case of Dray et al (case 10 in Table 2), which has full bilateral vocal fold motion, but electrical evidence of neuropathy, may represent a unique opportunity to follow an evolving CMT lesion. In view of the prevalence of bilateral vocal fold paresis in the disease, which probably represents the final end product of the neuropathy, we recommend caution with respect to the medialization procedure mentioned in that report.

Patients whose vocal fold paresis begins in childhood or infancy do not cope as well with vocal fold paresis. Of the 3 such patients described (cases 1, 4, and 5), 2 required tracheotomy in acute circumstances. It is a commonplace in otolaryngology that the smaller overall size of the pediatric airway tolerates any abnormality less well than that of the adult. Furthermore, early-onset paresis likely offers less chance for reinnervation and even partial recovery of abduction.

Sensory deficits are a part of the CMT disorders, considered late signs in general. We present the first assay of laryngeal sensation in a CMT patient. The stimulus is an air puff of measured pressure, the only laryngeal sensory modality presently able to be tested. The thresholds were normal on the left and only slightly elevated on the right, compared to those of healthy adults of the same age,[12] but demonstrated a clear and consistent difference between sides. Sensory testing is important in patients with CMT as a predictor of suitability for vocal fold lateralization procedures. Vocal fold lateralization to improve the airway has the potential to compromise the protective function of the larynx and permit aspiration. This potential is small in a patient with intact sensation, but may be prohibitive in a severely impaired individual.

Currently, vocal fold paresis is considered a hallmark of CMT2C. Although a preponderance of the reported cases belong to that designation, there are at least 2 cases (cases 3 and 11) that clearly do not. Both of these cases demonstrate some diaphragmatic dysfunction as well. Therefore, neither laryngeal nor respiratory muscle dysfunction is by itself sufficient for assignment of a case to CMT2C.

The overall incidence of vocal fold dysfunction in the CMT disorders is not known. That many patients carry a diagnosis of asthma for some time before diagnosis (eg, in the report of Dyck et al[2]), and that others are so well compensated that even the patient is sometimes unaware of his or her disability, suggests that many cases go unnoticed. This situation can only be remedied by clinical examination of the larynx of all patients with CMT. We urge this especially for children of families with known CMT, as in children the disability can be life-threatening.

CONCLUSION

Numerous reports demonstrate clearly that the motor neuropathy and probably the sensory neuropathy of CMT affect the vagus nerve, resulting in laryngeal dysfunction. This most often manifests as bilateral vocal fold paresis. Considered exclusively a feature of CMT2C, vocal fold paresis can apparently also occur in CMT1. In general, the clinical features of the bilateral vocal fold paresis of CMT in the adult are not as striking as idiopathic or iatrogenic bilateral paresis, probably because the progress of the disease is slow and allows periods of partial recovery of function. In contrast, affected children may develop acute respiratory compromise.

The prevalence of involvement of the larynx in CMT is unclear. We suspect that well-compensated cases have gone overlooked. Their identification would help clarify the role of laryngeal dysfunction in CMT. Ideally, all CMT patients should undergo laryngeal examination to identify gross vocal fold movement abnormalities. At the least, CMT patients with respiratory complaints, and children in known CMT kinships, warrant serious attention.

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COMPLETE BRANCHIAL FISTULA

CASE REPORT AND REVIEW OF THE LITERATURE

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Branchial anomalies, a result of aberrant embryonic development, are rarely seen in clinical practice. Lesions of the second branchial pouch commonly present as a neck lump or discharging sinus that may be complicated by infection. Clinical examination often reveals the lesion to be related to the junction of the upper two thirds and the lower one third of the sternocleidomastoid muscle. Branchial fistulas often present as a discharging sinus in the neck with the fistula tract extending upward within the deep neck tissue for a variable distance. A complete branchial fistula is one that has a defined internal opening in the tonsillar area and an external opening at the skin overlying the sternocleidomastoid muscle at the junction of the upper two thirds and the lower one third of the muscle. The incidence of such lesions is extremely rare. Surgical excision is the treatment of choice for branchial anomalies. We present the case of a patient who presented with a complete branchial fistula and discuss the clinical presentation and surgical management of such lesions, with a review of the relevant literature.

KEY WORDS — branchial anomalies, complete branchial fistula, surgical management.

CASE REPORT

A 20-year-old patient had had a right-sided discharging neck sinus since birth. His mother revealed that he had occasionally had a clear discharge from this sinus throughout his life. He did not complain of any erythema, swelling, or purulent discharge from the sinus. It was not brought to medical attention, as it was not of inconvenience to the patient. The patient was referred for an otolaryngology consultation by his military physician before conscription.

Physical examination revealed a small right-sided neck punctum at the skin overlying the anterior border of the sternocleidomastoid muscle, between the upper two thirds and the lower one third of the muscle. There were no clinical signs of inflammation; the neck was supple, with no palpable masses or cervical lymphadenopathy. The oral, nasal, and throat examination yielded essentially normal findings. There was no obvious punctum, swelling, or fistulous tract in the oral cavity. The tonsils were not enlarged.

A fistulogram (Fig 1) outlined a classic complete fistulous tract connecting the cutaneous punctum at the anterior border of the sternocleidomastoid muscle, proceeding deep in the neck, and ascending into the right side of the oropharynx. The results of biochemical investigations were normal.

SURGICAL PROCEDURE

Because this patient had a complete branchial fistula, a combined approach was used, ie, a transcervical approach followed by a peroral exploration for the parapharyngeal portion of the tract. A cervicotomy incision was made at the right lateral part of the neck. After injection of blue dye into the cutaneous punctum, the cervical portion of the fistula tract was carefully dissected from the surrounding structures, in continuity with the cutaneous punctum. The deep parapharyngeal portion of the tract was dissected free of surrounding structures and followed to the lateral wall of the right tonsillar fossa. A Davis-Boyle gag was inserted at this point, and a right tonsillectomy was performed. The parapharyngeal portion of the fistula tract, part of which was embedded within the substance of the right tonsil, was traced to the cervical portion of the tract and freed from the surrounding structures. The fistula tract in its entirety was delivered through the mouth. The right pharyngeal mucosal defect was repaired perorally.

The postoperative course of the patient was uneventful. He did not have any neurologic deficit after the operation. No complications arose as a result of the surgical procedure. Histologic analysis showed a fistula tract lined by benign squamous epithelium with a muscular wall.

DISCUSSION

Branchial fistulas and cysts, involving soft tissues of the head and neck, are uncommon anomalies of embryonic development. Branchial cleft cysts, fistulas, and sinuses may occur in any age group, but the first and second decades of life are the most common. Branchial cysts have been found to be more

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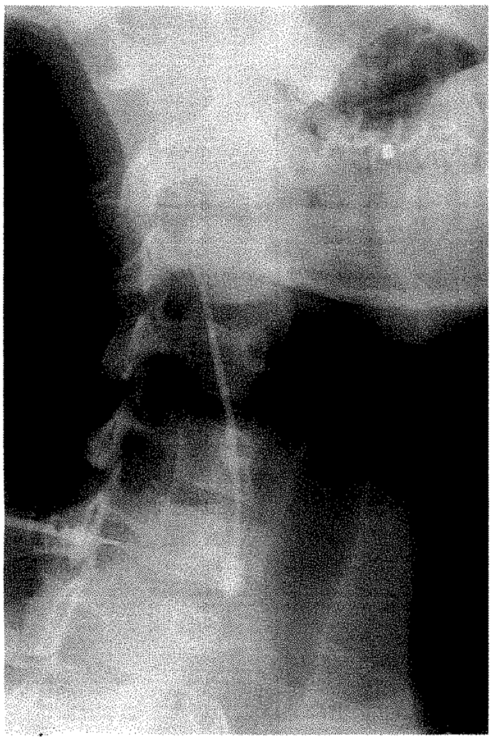

Fig 1. Preoperative fistulogram. Fistula tract is demonstrated with its external opening at skin overlying junction of upper two thirds and lower one third of sternocleidomastoid muscle, and its internal opening at right tonsillar bed.

prevalent (80.8%) than branchial fistulas (19.2%).[1] The presence of bilateral branchial anomalies has been reported in 1% to 30% of cases.[1]

Mesodermal condensations develop in the side wall of the embryonic pharynx, forming the branchial arches and their corresponding pouches. Derivatives of these arches and pouches develop into important structures of the head and neck region. During embryonic development, the second arch grows caudally, enveloping the third, fourth, and sixth arches and fusing with skin caudal to these arches, forming a deep groove (cervical sinus). The edges of this groove then meet and fuse, and the ectoderm within the fused tube disappears. Persistence of the ectoderm gives rise to a branchial cyst. A branchial fistula results from the breakdown of the endoderm, usually in the second pouch. The tract of the resultant fistula runs from the region of the tonsil, between the internal and external carotid arteries, and reaches the skin anterior to the lower end of the sternocleidomastoid muscle.

Second arch anomalies may take several forms. There may be only a simple sinus opening (usually only of pinpoint size) that extends up the neck for a variable distance. Branchial fistulas commonly present with persistent mucoid discharge from a skin opening in the neck, as seen in this patient. Other, more unusual methods of presentation have been documented. Second arch anomalies have been documented to present as parapharyngeal masses located in the supratonsillar fossa and extending to the lateral nasopharynx.[2] Exceedingly rarely, a branchial cleft anomaly may be found to be malignant on presentation.[3] A complete branchial fistula with external and internal openings is rare. In a retrospective study of 106 patients with branchial arch anomalies who presented to the Hospital for Sick Children in London between 1948 and 1990, only 2 complete fistulas were found.[4] The completeness of a fistula is diagnosed by a dye test in which methylene blue is injected through the outer opening and appears in the throat, or by a fistulogram, which can demonstrate the tract in its entirety. A negative preoperative outcome on the test might become positive under anesthesia because of muscle relaxation.[5] Occasionally, the fistula tract may be blocked by secretions or granulation tissue. Hence, the entire tract may not be delineated fully before operation. Computed tomographic scanning and magnetic resonance imaging of the neck are useful in delineating the branchial lesion and its relationship to surrounding neurovascular structures before any surgical intervention.

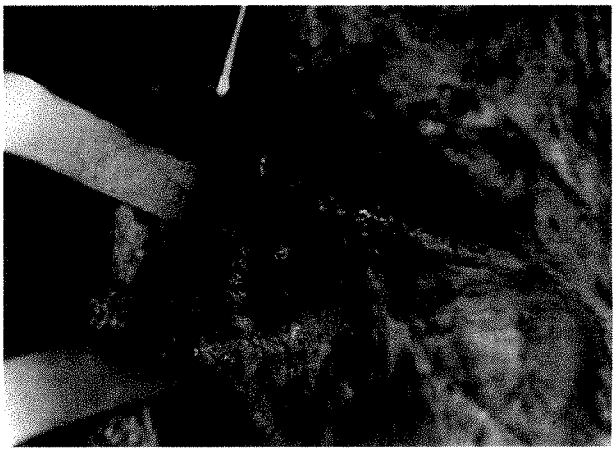

Fig 2. Dissection of fistula tract. Here, much of cervical portion of fistula tract has been freed.

Surgical excision of branchial cysts, fistulas, and sinuses is the definitive treatment. Several surgical approaches have been described for the management of a branchial fistula. A wide cervicotomy (hockey stick) incision remains the method of choice for excision of lesions of the second branchial cleft[1] (as described above). It allows for adequate exposure of neck structures for accurate dissection (Fig 2). In this patient, we combined it with a peroral approach to the parapharyngeal part of the fistula (Fig 3). This approach allowed for complete resection of the fistula tract. In our patient, the use of intraoperative dye allowed for identification of the intraoral opening of the fistula, which guided our resection. A pull-through pharyngotomy approach allowed the speci-

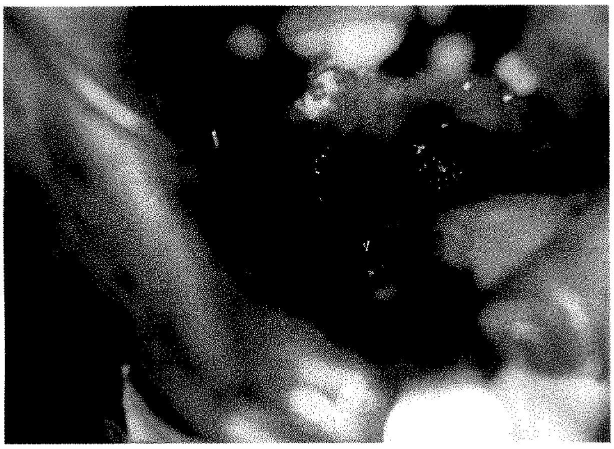

Fig 3. Peroral view of internal opening of tract as demonstrated by methylene blue dye in right tonsillar bed.

men to be delivered in continuity (Fig 4).

Other methods described include the stepladder approach and the stripping method. The stepladder approach was described in 1933.[6] The fistulous tract can be approached via a series of stepladder incisions, usually with the first encompassing the sinus opening and the second overlying the carotid bifurcation. The highest incision should be fairly large, to facilitate dissection of the deep parapharyngeal segment of the fistula. The skin incision in the stepladder approach is less extensive than the wide cervicotomy incision.

Alternatively, 1 or 2 small (stepped) incisions can be made in the cervical region for access to the infrahyoid portion of the fistula. Subsequently, the parapharyngeal portion of the fistula can be approached perorally after a tonsillectomy by means of a Davis-Boyle gag. The fistula, freed from surrounding structures, is then delivered through the mouth (pull-through technique).[5]

The stripping of a branchial fistula by passing the stripper inside the tract is a newer method, first described in 1977 by Taylor and Bicknell.[7] In 1991, Lee and Krishnan[8] described the use of this method in the excision of a fistula tract extending from the infra-auricular area to the supratonsillar fossa. It can be combined with a preliminary superficial parotidectomy with identification of the branches of the facial nerve, if the fistula tract is in close proximity to the facial nerve trunk. The advantage of this method lies in its simplicity and avoidance of extensive dissection. However, as it is essentially a blind procedure, it carries a higher risk of injury to surrounding structures. It is not a widely practiced procedure.

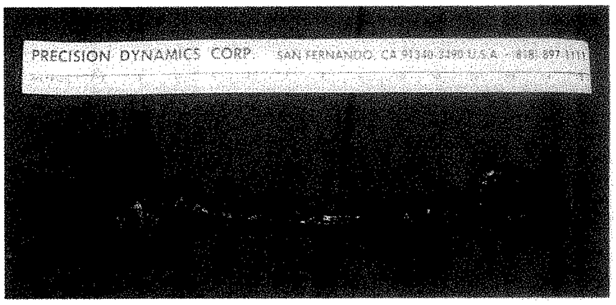

Fig 4. Pathological specimen. Excised fistula tract is shown in its entirety.

Ultimately, the choice of surgical approach is largely determined by the surgeon's preference and level of competence in the various methods. A certain surgical approach will be considered superior if it ensures complete excision of the fistula, entails minimal risk to surrounding structures, allows efficient closure of the deep pharyngeal mucosal defect resulting after fistulectomy, and has a good cosmetic result.

The recurrence rates of branchial cysts and fistulas after excision are not high. In 1996, Agaton-Bonilla and Gay-Escoda[1] found recurrence rates of 4% for branchial cysts and 8.6% for branchial fistulas (the difference was not statistically significant), for an overall recurrence rate of 4.9%.

CONCLUSION

A complete branchial fistula arising from the second pouch is rarely seen in clinical practice. Adequate preoperative evaluation should include clinical or radiologic assessment of the completeness of the fistula tract. Surgical excision is the treatment of choice in branchial anomalies. To date, various surgical techniques have been described in the approach to a branchial fistula, and the choice of an approach still remains very much the responsibility of the individual surgeon.

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AURICULAR EXOSTOSES AMONG THE PREHISTORIC POPULATION OF DIFFERENT ISLANDS OF THE CANARY ARCHIPELAGO

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It is generally believed that auricular exostoses are due to prolonged exposure to cold water, so their existence in ancient remains may inform us about economic activities in the past. This study was performed to analyze the prevalence of auricular exostoses among 150 pre-Hispanic individuals of different islands of the Canary archipelago. Individuals from Gran Canaria (6/26), Fuerteventura (1/15), and Tenerife (2/64), but not from El Hierro (0/45), showed auricular exostoses, thus supporting the hypothesis of a greater reliance on fishing and shellfishing in the former 3 islands.

KEY WORDS — auricular exostoses, ear canal, paleopathology, prehistoric Canary Islands.

Auricular exostoses are bony anomalies with an extensive base, mainly located on the floor of the external auditory canal, in contrast to auricular osteomata, which arise from the tympanomastoid and tympanosquamous sutures and project into the acoustic meatus. These last show a small, pedunculated base, and over time they narrow the lumen of the external auditory canal.[1,2] However, it is unclear whether they truly represent distinct entities.[3] Most clinicians and anthropologists prefer to use the term auricular exostosis to denote all such discrete lesions that occlude the external auditory canal.

Regarding pathogenesis, it seems that habitual exposure of the ear canal to cold water triggers an inflammatory reaction in the soft tissue that ultimately leads to osteogenic activity.[4,5] Therefore, the presence of exostoses has been linked to activities in cold water, such as diving or surfing. The water temperature has to be below 19°C, as is usually found in latitudes higher than 30°. Several groups have analyzed the prevalence of auditory exostoses in ancient population groups, such as prehistoric Chileans,[6] Mesolithic inhabitants of Yugoslavia,[7] ancient Romans,[8] and other population groups,[9,10] as the finding of these exostoses may provide information about economic activities.

In a previous study,[11] we found a high prevalence of auditory exostoses among the pre-Hispanic inhabitants of Gran Canaria buried in the coastal regions, in sharp contrast to the prevalence in individuals buried in the central highlands. This observation suggests that different economic activities were performed by the two population groups, although diachronic differences cannot be fully excluded.

The aim of the present study was to analyze the prevalence of auditory exostoses among individuals of different islands of the archipelago, comparing them with the results from 3 coastal burial sites from Gran Canaria that were not included in previous studies.

MATERIALS AND METHODS

We analyzed a total of 150 crania of adult pre-Hispanic individuals from different islands of the Canary archipelago. Sex was established by the Krogman and Iscan[12] criteria. The sample included the following.

1. Fifteen crania from Fuerteventura (11 men and 4 women) housed in the Museo Canario (Las Palmas).

2. Twenty-six crania from Gran Canaria (16 men and 10 women) from individuals buried at 3 different coastal sites (Crusesitas, Draguillo, and La Pardilla). These crania are housed in the Museo Canario.

3. Forty-five crania from El Hierro (23 men and 22 women). Three of the 45 crania are housed in the Museo Canario; the others were recently excavated by one of us (J.V.V., Lajura site) and are currently deposited in the Cabildo Insular de El Hierro. This collection constitutes the majority of the currently known pre-Hispanic crania from this island.

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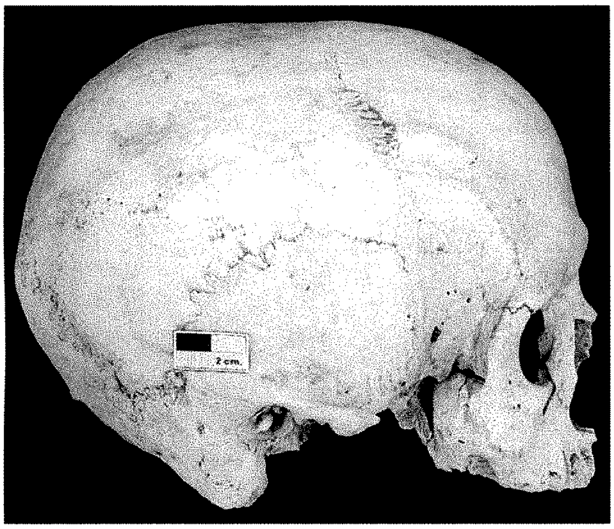

Case of auricular exostoses in pre-Hispanic individual from Gran Canaria.

4. Sixty-four crania from Tenerife, 20 of which are housed in the Museo Canario; the sample is composed of 35 crania from men (13 from the Museo Canario), 25 crania from women (7 from the Museo Canario), and 4 crania from individuals of unknown sex. They are from individuals buried in caves located in Barranco de la Orchilla, Barranco del Infierno, La Orotava, La Laguna, Vilaflor, and Ucanca. Forty further crania were found in Tegueste and are kept in the Instituto Cabrera Pinto (La Laguna, Tenerife). Finally, 4 additional crania belong to the collection of the Museo de Güímar (Tenerife); they were found at Cueva Bermeja, a coastal site; 2 are from men and 2 are from women.

The antiquity of the samples is unknown, except for the samples from Lajura, which are from 2120 BC; there are other data for remains from Gran Canaria and Tenerife (not included in this study), yielding figures ranging from 2500 BC to 547 BC.[13,14]

The external auditory canal was examined with the naked eye for the presence of exostoses, and records were made of their morphology[1] and location, whether they were unilateral or bilateral, and to what extent they occluded the auditory canal (less than one third, one third to two thirds, or more than two thirds).[6]

RESULTS

We found 6 cases of auricular exostoses in the sample from Gran Canaria (23.08%; see Figure), and only 1 among the 15 cases from Fuerteventura (6.67%); no cases were recorded among the population from El Hierro, and 2 cases (3.13%) were observed among the crania from Tenerife. These differences are statistically significant ($p < .05$).

The single case from Fuerteventura was in a man, was located posteriorly, and occluded less than one third of the external auditory canal. Five of the 6 cases of exostoses from Gran Canaria were observed in men ($p > .1$). The distribution of the exostoses in relation to sex and age at death is shown in the Table.

AURICULAR EXOSTOSES DISTRIBUTED BY SIZE, SEX, AND AGE AT DEATH

Involvement of EAC	*Age (y)* 17-25	25-35	35-45	>45
$<1/3$	1 (1) F	1 (0) GC	2 (2) GC, TF	1 (1) GC
$1/3$ to $2/3$			1 (1) GC	
$>2/3$		1 (1) GC	2 (2) GC, TF	

Numbers of male subjects are in parentheses.

EAC — external auditory canal, F — Fuerteventura, GC — Gran Canaria, TF — Tenerife.

All 3 cases from Crusesitas (Gran Canaria) occluded less than one third of the external auditory canal; the 2 cases from Draguillo occluded more than two thirds of the canal, and the case from Pardilla occluded between one and two thirds of the canal. One of the cases from Tenerife occluded more than two thirds of the canal, and the other, only one third.

DISCUSSION

North African people colonized the Canary archipelago about 2,500 years ago.[15] Although the different islands of the archipelago are related to each other, the archaeological remains show marked differences between islands, supporting the hypothesis of several arrivals of related tribes or ethnic groups, perhaps separated by dozens or even hundreds of years. These people adapted their economy to the different geographic and climatic conditions of the different islands, and demographic growth was different in each of them. Indeed, marked differences exist between La Palma, with its dense forests and abundant rainfall, and Fuerteventura, with its desert climate. The two central islands, Tenerife and Gran Canaria, are currently the two main islands of the archipelago; together, they contain more than 80% of the total population. Probably, this was also the case in the past, although Gran Canaria was more densely inhabited than Tenerife. The prehistoric Gran Canaria was a proto-urban, strongly hierarchized society whose economy was strongly dependent on agriculture. But chroniclers have written that fishing and shellfishing were also important economic activities[16] — an assertion supported by archaeological findings.[17]

We found that 23.1% of the population from Gran Canaria showed auricular exostoses. The individuals analyzed in this study were buried in coastal sites, so the results are in accordance with the previous observations, although other investigators, analyzing the tumulus El Agujero (coastal Gran Canaria), found exostoses in 22 of 34 individuals.[18] The prevalence of exostoses reported here is similar to that observed in other population groups.[6,9,19] As stated earlier in this paper, except for some investigators,[20] there is general agreement concerning the relationship between the formation of exostoses and prolonged exposure to cold water. Thus, it is possible that the exostoses found in the inhabitants of Gran Canaria are related to fishing activities.

In contrast to the results observed in Gran Canaria, there is a striking absence of exostoses in the pre-Hispanic population from El Hierro. Two different possibilities exist to explain these results: either the economy of the inhabitants of this island did not include fishing or diving, or the water temperature was not as cold as in Gran Canaria. The islanders from El Hierro consumed large amounts of limpets and other marine mollusks. Indeed, huge accumulations of shells can be seen in archaeological sites on both the northern and southern shores of the island.[21] These shell accumulations are by far more important than those observed in Gran Canaria, despite the enormous difference in population between the two islands. (El Hierro was sparsely inhabited.) Moreover, there are also remains of fish consumption, although mainly of species that live in the shallow waters and ponds of the volcanic shore of the island. Limpets and the other mollusks consumed live in the rocks that become exposed during low tide, so it is possible that consumption of these amounts of shellfish did not require diving. Also, the speculative possibility that only some people (whose skulls were not analyzed here) were involved in fishing remains open.

Differences in water temperature, which may be as great as 2°C or 3°C in different parts of the archipelago, may also account for the absence of auricular exostoses among the pre-Hispanic population of El Hierro. El Hierro is located in the (south)western part of the archipelago, where the influence of the Canary cold stream is less pronounced than in the east.

Tenerife is the largest island of the archipelago. The pre-Hispanic inhabitants, who offered — as in Gran Canaria — a tenacious resistance against the Spanish conquest, based their economy mainly on goats and sheep, complemented with some agriculture. Only 2 cases of 64 show auricular exostoses. As with El Hierro, this fact is quite difficult to explain. Consumption of shellfish was probably less important: shell accumulations are much less than in El Hierro. The water temperature is similar to that of Gran Canaria. In any case, the sample from Tenerife, although including the totality of skulls housed at Museo Canario, Museo de Güímar, and Instituto Cabrera Pinto, probably represents a minority of the total number of preserved skulls from the pre-Hispanic population of this island. However, there is no reason to suppose that the skulls analyzed in this work do not represent a random sample of the total population.

Very little is known about prehistoric Fuerteventura. We have here analyzed the only 15 skulls preserved from the pre-Hispanic people housed at the Museo Canario, and auricular exostosis was present in 1 case. The Canary cold stream bathes the coast of the island, which is relatively flat and desertic. The water temperature is lower than that of the other islands analyzed in this study; it is probable that shellfishing and fishing constituted an important part of the economy of the sparse population, although goatherding was the main economic activity.

In conclusion, we have found striking differences in the prevalence of auricular exostoses among the pre-Hispanic inhabitants of different islands of the Canary archipelago, which may be attributable to different economic patterns and perhaps also to differences in water temperature.

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IMAGING CASE STUDY OF THE MONTH

MIDDLE CRANIAL FOSSA CHOLESTEATOMA FOLLOWING BLAST TRAUMA

ABRAHAM GOLDFARB, MD
MENACHEM GROSS, MD
RON ELIASHAR, MD
JOSEF ELIDAN, MD

JERUSALEM, ISRAEL

INTRODUCTION

Blast trauma can have devastating effects on the ear, including tympanic membrane perforation, ossicular chain damage, perilymphatic fistula, and cochlear or vestibular injury.[1] Blast trauma can also be the cause of cholesteatoma, typically in the middle ear cavity. Blast injury causing an intracranial cholesteatoma has not been described thus far. A case of middle cranial fossa cholesteatoma occurring 29 years after blast trauma is presented.

CASE REPORT

A 50-year-old patient was referred to our department in 1998 with complete right facial paralysis. This generally healthy man had been exposed to blast trauma during combat 29 years earlier. He had suffered shrapnel injuries to the right flank, bilateral tympanic membrane perforations, and profound right sensorineural hearing loss at the time. Myringoplasty had been performed 12 months after his injury and was successful on the left ear. Myringoplasty of the right tympanic membrane, performed 1 month later, had failed. The perforation was eventually repaired on revision surgery 1 year later. The patient had been generally well and without otologic complaints since that time.

In May 1994, the patient noticed very mild ptosis of his right upper eyelid. He was examined by his family physician, who decided that no further evaluation or treatment was necessary because of the minimal findings. Indeed, the ptosis had completely resolved within 3 months. Four years later, sudden complete peripheral paralysis of the right facial nerve developed. The patient had no complaints of aural pain, discharge, or vertigo. An initial diagnosis of Bell's palsy was made by the family physician. However, a computed tomography scan demonstrated a sclerotic right temporal bone with a soft tissue mass destroying the petrous apex and invading the right internal auditory canal (Fig 1). The differential diagnosis at that time included epidermoid cyst, cholesteatoma, and geniculate ganglion schwannoma, although the lesion did not enhance with contrast (thus decreasing the likelihood of schwannoma). An old

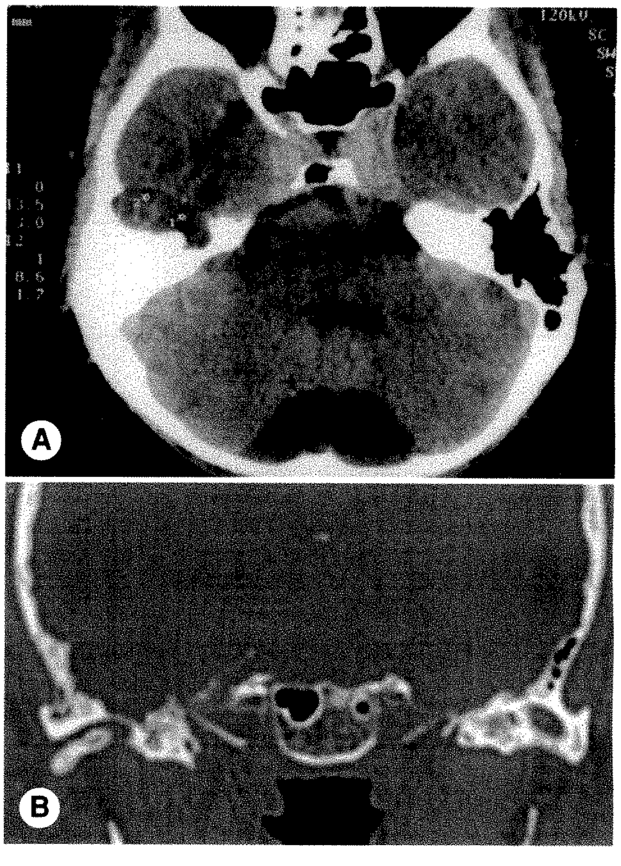

Fig 1. Computed tomography. **A)** Axial view with contrast demonstrates sclerotic right mastoid bone with bone destruction and soft tissue mass displacing temporal lobe. **B)** Coronal view with bone window demonstrates extent of damage to right petrous bone.

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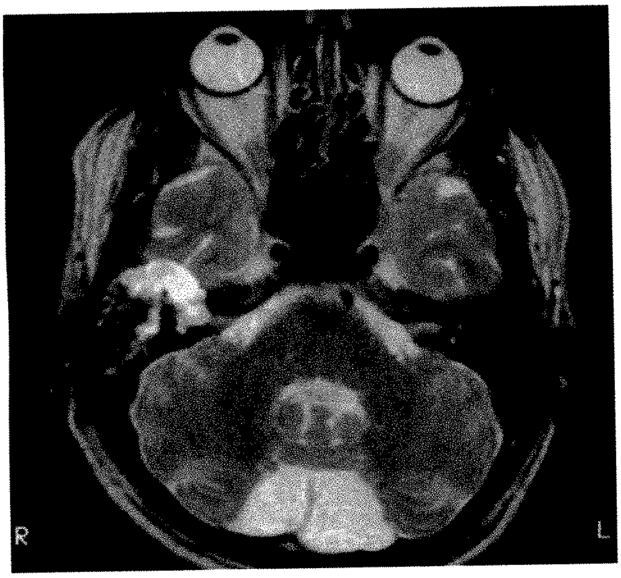

Fig 2. Magnetic resonance imaging demonstrates mass with very bright signal on T2-weighted image. Mass appears to be in contact with seventh and eighth cranial nerves in lateral part of internal auditory canal.

fracture of the petrous bone extending into the tegmen mastoideum was also identified.

Magnetic resonance imaging demonstrated an oval lesion destroying the anterior border of the petrous pyramid and extending below the temporal lobe and displacing it upward. The lesion appeared to involve the geniculate ganglion and the upper lateral portion of the internal auditory canal. The lesion appeared nonhomogeneously bright on T1-weighted scans, and an intense signal was seen on T2-weighted images (Fig 2). There was no significant enhancement by gadolinium. The differential diagnosis offered by the radiologists included cholesteatoma, cholesterol granuloma, and an atypical epidermoid cyst.

On examination, the patient had a complete peripheral right facial paralysis with Bell's phenomenon. The rest of the neurologic findings were normal. Otoscopy revealed lateralization of the right tympanic membrane, which was slightly thickened, without a perforation or signs of cholesteatoma. The left tympanic membrane was slightly retracted, with no other apparent disease. The Weber test localized to the left.

Audiometry results showed profound sensorineural hearing loss on the right and mild to severe sensorineural hearing loss between 3 and 8 kHz on the left. Tympanometry revealed a type A curve on the left and a type B curve on the right (probably due to the tympanic membrane lateralization).

The patient underwent mastoidectomy combined with a middle cranial fossa (MCF) approach. This combination was selected because it was suspected that the lesion had originated in the mastoid air cells.

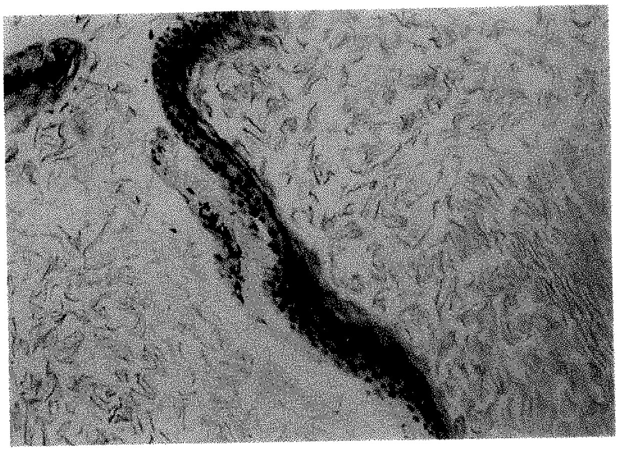

Fig 3. Pathological specimen (H & E, original ×20). Cyst wall is made up of keratinizing squamous epithelium and keratin material.

The tympanic and mastoid cavities were well aerated and free of cholesteatoma and of infection. The tegmen mastoideum was absent, and the root of the cholesteatoma extended from there to the MCF. There was no adhesion between the cholesteatoma and the seventh or eighth nerves, and separation was achieved with relative ease. The pathology findings were consistent with the diagnosis of cholesteatoma (Fig 3).

After the operation, there appeared to be improvement in eye closure and in gross movements of the facial musculature. However, 3 days after surgery, the function of the seventh nerve had deteriorated and there was complete paralysis. The patient remained hospitalized for observation, initially in the intensive care unit and later on the ward. He was discharged 1 week later.

Twelve months after surgery, the patient has recovered function of his facial nerve, to grade II-III by the House-Brackmann classification. On examination, the tympanic membrane appears thickened and lateralized; however, there is no evidence of cholesteatoma and there is no evidence of recurrent disease on computed tomography or magnetic resonance imaging.

DISCUSSION

Middle ear cholesteatoma is a well-documented complication of blast trauma. Kronenberg et al[2] report a series of 147 ears exposed to blast injury. Of their patients, 8% suffered from middle ear cholesteatoma and 1.8% from confined (pearl) lesions behind an intact eardrum. Wolf et al[3] describe 210 ears exposed to blast injury; 2.8% had pearl lesions and 4.8% had invasive cholesteatoma. This group recommends postponement of closure of tympanic membrane perforations for 1 year after trauma. They also recommend a minimum follow-up period of 4 years for detecting late complications. Freeman[4] describes the connection between temporal bone fractures and cholesteatoma, and suggests that longitudinal fractures (which are considered extralabyrinthine) and mixed fractures provide an origin for epithelial invasion of the middle ear. He claims that callus formation usually provides a barrier to the ingrowth of epithelium from the external auditory canal. However, entrapment of epithelium before callus formation, displacement of suture lines and of bone fragments, and tears of the tympanic membrane are possible mechanisms for the creation of a cholesteatoma.

Posttraumatic cholesteatoma of the external auditory canal has also been described.[5] A search of the literature, however, did not reveal any description of posttraumatic cholesteatoma involving the MCF.

In the present case, the manifestation of cholesteatoma was facial nerve paralysis that appeared for the first time 25 years after injury. There were no symptoms of ear infection and no signs of cholesteatoma. The late onset of symptoms is similar to the previous descriptions by Freeman[4] and by McKennan and Chole,[6] who describe cholesteatoma occurring 12 and 7 years after injury, respectively. Bottrill[7] reports a posttraumatic cholesteatoma presenting 24 years after a fall trauma with fractures in the temporoparietal region that necessitated insertion of a skull prosthesis. In that case it was demonstrated that epithelium was implanted at the fracture line.

The operative findings in our patient demonstrated that the cholesteatoma had indeed originated at the fracture line in the tegmen mastoideum. It was located in the MCF, and the middle ear and the mastoid cavities were preserved. The most probable mechanism for these findings is implantation of epithelium through the fracture line with subsequent callus formation. The epithelium continued to grow in the MCF, but the callus hindered growth into the middle ear. Worthy of note is the fact that in all other reported cases, aural discharge and infection were present at diagnosis. Our patient presented only with facial paralysis, and his disease escaped detection in 1994, presumably because imaging studies were not performed.

The case presented in this report demonstrates that the insidious nature of posttraumatic cholesteatoma requires alertness on the part of the attending physician. Imaging studies in unexplained cases of peripheral facial palsy may prove to be invaluable, especially when there is a history of previous trauma, no matter how distant.

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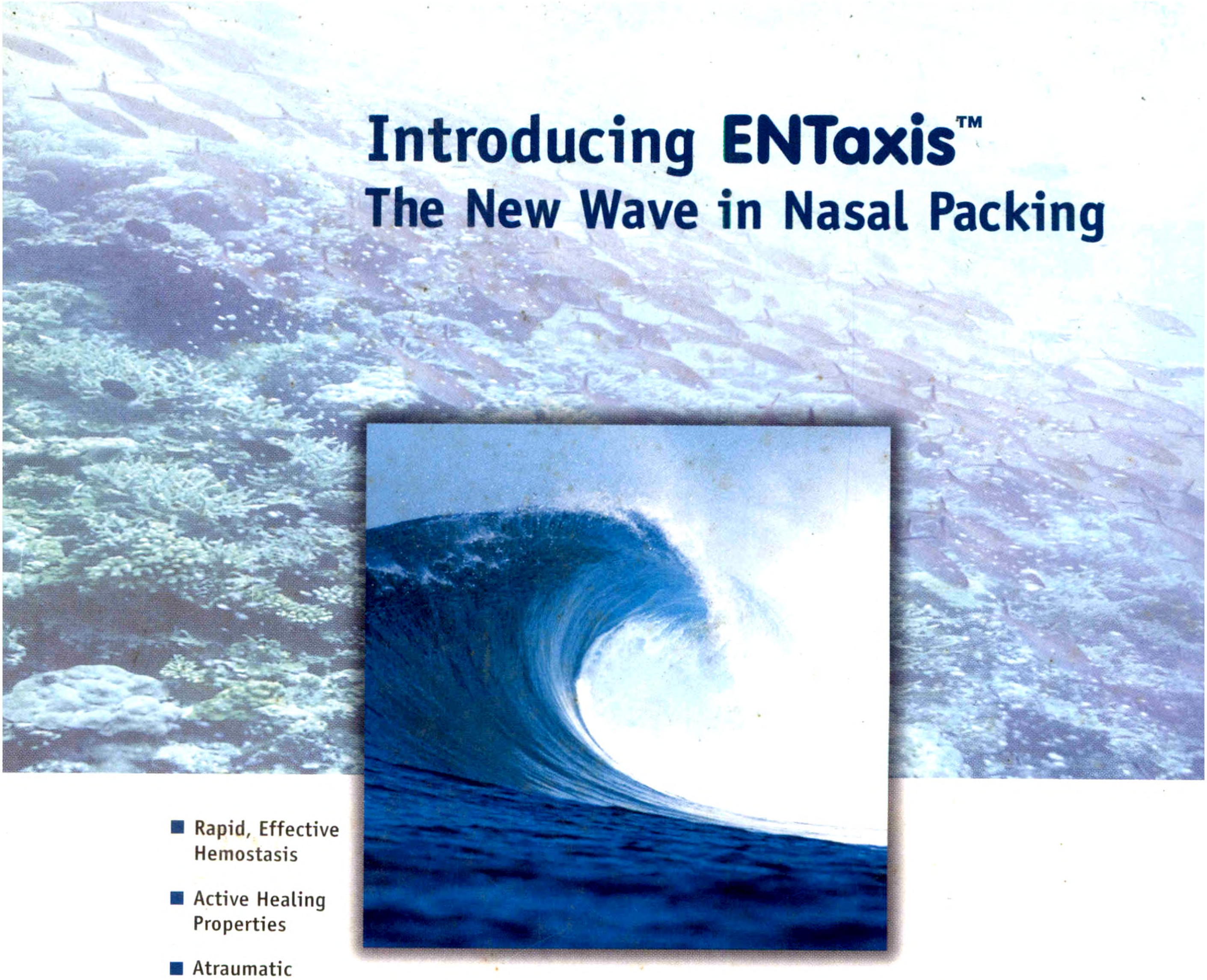

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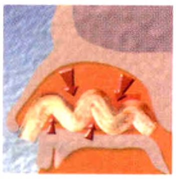

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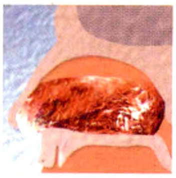

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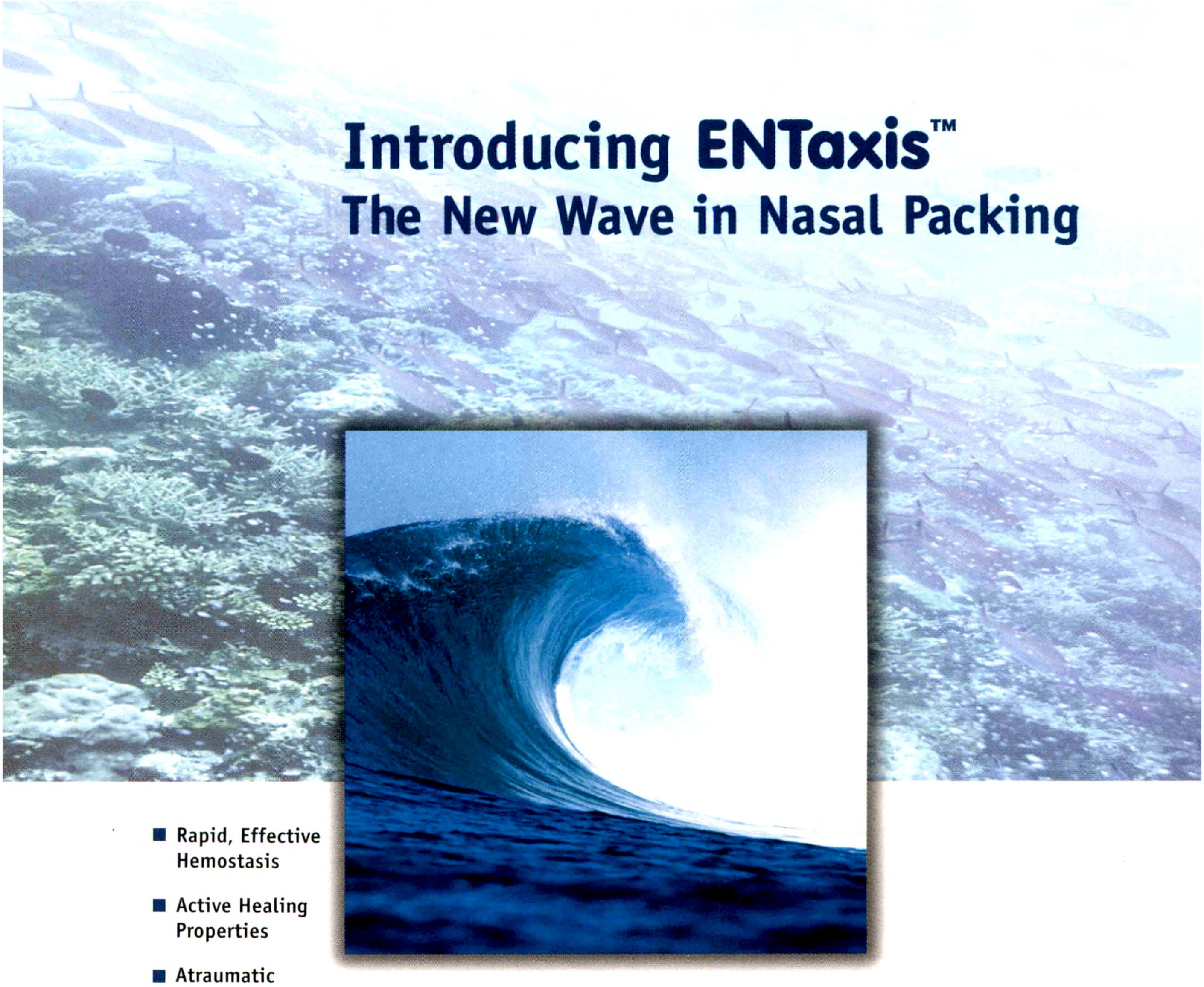

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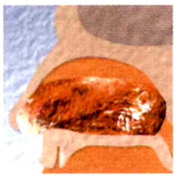

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For further information regarding these courses, contact Henry T. Hoffman, MD, Dept of Otolaryngology,
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REVISION THYROPLASTY

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ROCHESTER, MINNESOTA

To document surgical techniques for performing a revision thyroplasty operation and to describe the subjective and objective results of these operations, I undertook a retrospective review of all patients who have undergone thyroplasty in my practice. I identified patients who required revision of a previous thyroplasty and evaluated preoperative and postoperative video and audio analyses of the voice. I identified the surgical steps necessary for successful completion of a revision operation and noted any special findings made at surgery. Charts were reviewed and tabulated for reasons for failure of the first operation, efficacy of the second surgical approach, specific anatomic findings made at revision surgery, and types of surgery available for revision. Of 625 total thyroplasties in 331 patients, revision operations accounted for 61 operations in 48 patients. All thyroplasty operations were found to be revisable or reversible. Objective voice analysis for 35 operations in 32 patients revealed a statistically significant improvement in shimmer in women, and in harmonics-to-noise ratio in all patients. Subjective voice improvement occurred in 80% of patients after revision surgery. I conclude that revision thyroplasty is possible with all thyroplasty types, giving significant improvement in both shimmer (women) and harmonics-to-noise ratio (all patients). Although most voices are still abnormal on postoperative objective voice analysis, 80% of patients are subjectively improved.

KEY WORDS — revision, thyroplasty.

INTRODUCTION

Standard laryngeal framework operations include the Isshiki thyroplasty types I through IV and the arytenoid adduction.[1,2] Added to this is the arytenoid fixation technique,[3] which I have used for arytenoid dislocations and fractures and for failed attempts at arytenoid adduction. These operations have also been purported to be able to be revised or reversed if the vocal result was not satisfactory. In addition, the type I thyroplasty has been used in the early months after a vocal fold paralysis to compensate for glottic insufficiency in the face of possible recovery of the paralysis. In the latter case, it was supposed that the thyroplasty could be reversed if nerve recovery ensued and glottal function warranted it. However, data on these possibilities are lacking.

Laryngeal framework surgery as a whole is unique in its approach to the vocal generator as an active, functional entity and not just a static structure in the way of the lungs' air escape. As such, more attention is placed on the entire organ and the physiology of voice production than on the stationary position of one vocal fold.[4-12] Type I thyroplasty displaces the anterior membranous vocal fold medially, but still, that practice contrasts with the injection of foreign material or fat into the vocal fold. The latter changes the vocal fold's mass, stiffness, and, most important, aerodynamic shape, and violates the mucosal surface if done transorally.[13] The type II thyroplasty (vocal fold lateralization)[14] is rarely used, its counterparts being the suturing techniques for arytenoid lateralization, and the arytenoidectomy operations based on early work by Thornell[15] and Woodman.[16] The type III (vocal fold shortening)[17] and type IV (vocal fold lengthening)[18] operations have counterparts in the literature, but none that affect the vocal folds as completely as these. They are meant to relax or tense the vocal fold, respectively, thereby changing the glottal resistance, vocal fold shape, and fundamental frequency of the voice.

Various attempts have been made in the past to produce arytenoid medialization to make the posterior cartilaginous glottis more competent, but none are yet as predictable as the adduction technique.[1] Newer arytenoid fixation techniques[3,19] have been shown to medialize the arytenoid cartilage as well, but I find these operations are more difficult to perform. In addition, they negate any possible postoperative joint mobility, since the cricoarytenoid joint is essentially destroyed. Combinations of the arytenoid adduction and the type I thyroplasty[20-22] are used to compensate for incompetence of the entire length of the glottis. Other combinations are also possible, if one keeps in focus the glottic functional compensation required by the patient's deficit.

The necessity for either revising or reversing one of the framework operations stems from intraoperative misjudgment, edema, or hematoma, or from postoperative "resetting" of the laryngeal muscles by the patient. If postoperative examination suggests that

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TABLE 1. TOTAL THYROPLASTY OPERATIONS AND REVISIONS

Operation and Action on Vocal Fold	*Total No.*	*Total No. Revisions*
Type I: anterior medialization	406	45 (11.1%)
Type II: lateralization	10	1 (10%)
Type III: shortening	8	3 (37.5%)
Type IV: lengthening	45	5 (11.1%)
Arytenoid adduction: posterior	142	5 (3.5%)
Arytenoid fixation: posterior fixation	17	2 (11.8%)

the vocal result may be improved further, another attempt may be reasonable. Possible factors prohibiting revision or reversal operations include postoperative scarring and inappropriate patient expectations.

MATERIALS AND METHODS

From January 1, 1987, to October 1, 1999, I performed 625 thyroplasty operations on 331 patients. Forty-eight patients underwent 61 operations for revision of their previous surgery (14.5% of patients, 9.8% of operations), the expectation being vocal improvement in 47 and airway improvement in 1. The breakdown by operative type and number of revisions is given in Table 1.

All patients had preoperative and postoperative evaluations of laryngeal function, including high-quality voice recordings and/or videostrobolaryngoscopy. Voice recordings were obtained with an AKG D330T microphone and a high-quality Onkyo tape machine with metal quality audiotape cassettes for storage. A microcomputer paired with the CSL 4300 (Computerized Speech Lab, software version 5.0, Kay Elemetrics Corp) captured and analyzed the audio data. Videostrobolaryngoscopy was performed with either a Brüel and Kjaer stroboscopic unit or a Kay Elemetrics RLS 9100, paired with an Olympus ENF L3 nasopharyngoscope or a Nagashima 70° rigid telescope. A Sony EVO 9800 Hi-8 videocassette recorder stored all the video data.

For voice analysis and determination of frequency, jitter, shimmer, and harmonics-to-noise ratio (HNR), subjects were asked to produce a steady-state vowel /a/ for as long as possible at a comfortable volume and pitch while the acoustic signal was recorded. Approximately 1 second of the waveform from the midportion of each vowel prolongation was used to calculate jitter (cycle-to-cycle frequency variability), shimmer (cycle-to-cycle amplitude variability), and HNR (ratio of energy in the periodic and aperiodic signal components.)

SURGICAL TECHNIQUES

All patients seen by me initially (36/49) underwent standardized thyroplasty techniques as previously described in other articles: type I thyroplasty,[5,23,24] type II thyroplasty,[14] type III thyroplasty,[17] type IV thyroplasty,[18] arytenoid adduction[20-22] with posterior window modification,[25,26] and arytenoid fixation.[3] All of my operations, both original and revision, were performed with local anesthesia and intravenous sedation. The following are short descriptions of the individual revision operations.

Revision of Type I Thyroplasty. After splitting of the strap muscles in the midline, the thyroid notch, inferior thyroid cartilage border, and vocal fold level are marked with gentian violet. It is usually necessary to bare the entire thyroid ala in order to see exactly where the previous operation may have failed in properly placing the rectangular cartilage window. All revision operations have been done on previously placed silicone implants, and no experience has been gained with either of the other two presently popular materials for anterior vocal fold medialization: hydroxylapatite (Vocom, Smith Nephew) and Gore-Tex (Gore). With silicone implants, a thick capsule of fibrous tissue is formed over the implant, and the sutures holding this are easily identified. The external capsule with the silicone is then removed.

If the previous type I thyroplasty preserved the cartilage window intact, then the internal fibrous tissue holding the depressed cartilage in place at the edges of the window is incised around the cartilage and down to muscle (No. 11 blade or 0.8-mm cutting drill). This incision sufficiently frees the cartilage, scar, and mass and allows revision of the previous thyroplasty. All tissue on the external surface of the depressed window cartilage is left intact.

If the type I window cartilage was removed at the initial operation, then all that is left after silicone removal is the thick internal fibrous tissue and scar. In this case, the fibrous tissue is incised in the shape of a properly placed window (just opposite the vocal fold), with this scar preserved for future medialization and lateral vocal fold stability. Complete reversal of this medialization is probably not possible, but may be attempted by suturing the internal fibrous tissue slightly lateral to the level of the original inner perichondrium of the surrounding thyroid cartilage.

A word about complete "reversal" of the type I thyroplasty is necessary at this point because of controversy among laryngologists over this possibility. "Reversal," as used here, means bringing the previously depressed cartilage back to the level of the surrounding thyroid cartilage, or restoring the inner perichondrium overlying the lateral vocal fold to its original position, level with the surrounding inner perichondrium of the thyroid cartilage. Reversal is possible with the window cartilage in place, but dissection is necessary at the fibrous tissue internally. In some cases, suture fixation of the window cartilage laterally may be necessary. Fortunately, of 45 total revision or reversal type I thyroplasties, revisions (42/45) were much more common than reversals (3/45).

Measurements of the original silicone implant are taken to identify the original cartilage–fibrous tissue depth, and either the old block is reused or a new block is carved to medialize the vocal fold at a more correct depth. Preoperative videostrobolaryngoscopy may help determine this depth. If the original cartilaginous window is in the wrong location, a new, properly placed window is made, and a new silicone block is fashioned to fill the original cartilage defect *and* hold the soft tissue of the new window at a proper depth (intraoperative videolaryngoscopy). Finally, the silicone is secured to the surrounding thyroid cartilage (4-0 nylon sutures), the strap muscles are reapproximated, and skin is closed over a small Penrose drain. Postoperative antibiotics are given for 1 week.

Revision of Type II or III Thyroplasty. Revisions of the type II and III thyroplasties are similar and are, therefore, covered together. Type II and type III thyroplasties require vertical cuts through the thyroid cartilage either in the midline (midline type II) or 7 to 8 mm lateral to the midline (lateral type II, type III). Stabilization of the cartilage pieces is of paramount importance for successful completion of the surgery.

The type III thyroplasty attempts to lower the vocal pitch by relaxing the anterior vocal fold and resetting the anterior commissure posteriorly. With the bilateral type III, both the supraglottic and subglottic muscle forces destabilize the anterior cartilaginous segment and, thus, the anterior commissure. Suture, silicone, or wire mesh stabilization is difficult but necessary. Revising the type III is relatively easy. The resulting positions of the thyroid cartilage pieces are best stabilized with wire mesh if the anterior commissure needs to be pulled anteriorly. For further posterior placement, the lateral thyroid cartilage pieces may require more trimming, and silicone, permanent sutures, or wire mesh may be used for stabilization.

Two types of type II thyroplasty exist: lateral and midline. A lateral type II attempts to lateralize and stretch the ipsilateral vocal fold, and has been used in women with bilateral vocal fold paralysis.[14] The midline type II is a recent addition to the thyroplasty family and has thus far been used in patients with adductor spasmodic dysphonia.[27] The midline type II may be revised by varying the distance between the two thyroid cartilage halves and restabilizing them with silicone or miniplates.

Revision of Type IV Thyroplasty. The type IV thyroplasty is the easiest of all thyroplasties to perform, and the easiest to revise. It stretches the vocal folds and tenses them by approximating the thyroid and cricoid cartilages. It is used primarily to compensate for symptomatic superior laryngeal nerve paresis or paralysis.[18] Revising a type IV thyroplasty requires incising the scar tissue connecting the thyroid and cricoid cartilages, and cutting the nylon sutures placed at the first operation. The cricothyroid distance is then modified while testing the voice, and new sutures are placed (2-0 nylon).

Revision of Arytenoid Adduction and Arytenoid Fixation. The arytenoid adduction medializes and stabilizes the arytenoid cartilage, closing the posterior glottis. It may be revised by direct reoperation and conversion to an arytenoid fixation if the posterior glottis remains open after initial operation. If the arytenoid cartilage is over-rotated postoperatively, it is easier to perform a direct laryngoscopy and laser removal of the vocal process than to reoperate directly.

The arytenoid fixation is used by the author for arytenoid dislocations and fractures,[3] after a previous arytenoid adduction failure, or if exploration of the cricoarytenoid joint intraoperatively shows complete ankylosis. Arytenoid fixation requires opening the entire cricoarytenoid joint and suturing the arytenoid cartilage *with the vocal process* to the medial cricoarytenoid joint. The point of suturing is 5.0 mm medial to the lateral joint capsule.[28]

RESULTS

A total of 61 revision thyroplasties were performed in 48 patients between January 1, 1987, and October 1, 1999. The history of antecedent disease, reasons for failure of the first operation, and the final outcome were evaluated for each patient. Sixteen of the 48 patients were unsuitable for study, having not completed preoperative and postoperative videostrobolaryngoscopy with audio recording. Ten of these have been lost to follow-up, and 6 have too recently had revision surgery to have postoperative analysis. The remaining 32 patients underwent a total of 35 revision operations. Their preoperative and postoperative voice analysis results are given below.

Voice analysis included jitter, shimmer, and HNR. Changes in the mean values from preoperative to postoperative, both for initial surgery (when possible) and for revision operations, were calculated and are shown graphically in Figs 1-3. The mean jitter was found to be better postoperatively, but the change was not statistically significant when measured for the entire group or for men and women separately

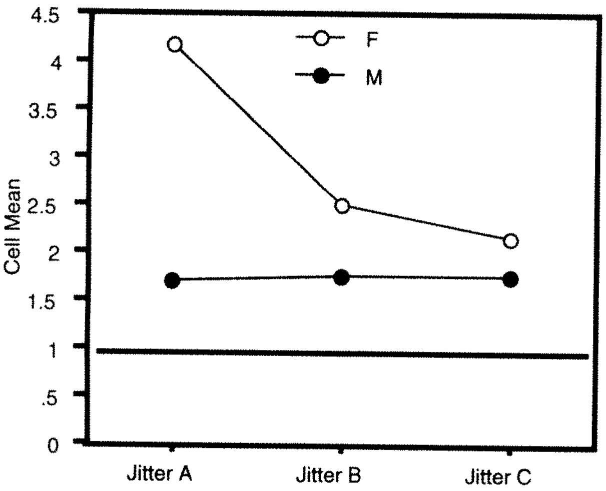

Fig 1. Mean (n = 35) jitter before operation (A), after initial thyroplasty (B), and after revision thyroplasty (C), separated by sex.

(Fig 1). As expected, differences in vocal frequency before and after revision surgery were also not significant. However, the postoperative decrease in shimmer (Fig 2) and the postoperative increase in HNR (Fig 3) were significant (paired *t*-test) for both the entire group and for the individual sexes.

The impetus for a revision thyroplasty came from the patient's desire to have a better voice or airway after the initial thyroplasty attempt. The patients were divided into 4 categories of subjective vocal symptoms: improved but still seeking a better voice, improved voice with subsequent worsening, no postoperative improvement, or worsening from the preoperative state. The same categories were used after revision operation, and Table 2 outlines the preoperative and postoperative subjective voice data for the revision thyroplasty. The final analysis shows that patients were subjectively improved after 28 of 35 revision operations (80%).

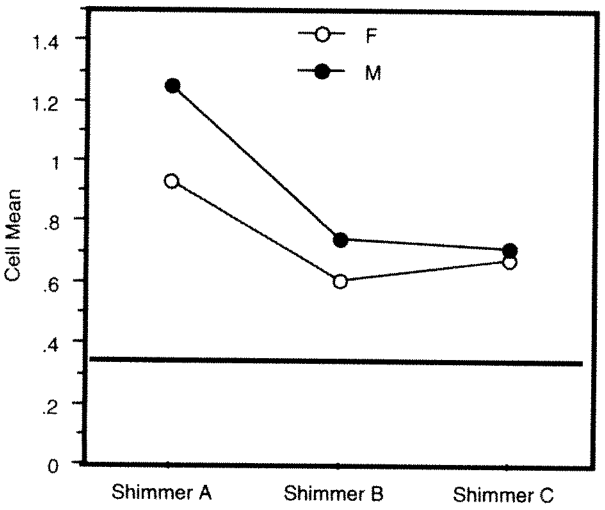

Fig 2. Mean (n = 35) shimmer before operation (A), after initial thyroplasty (B), and after revision thyroplasty (C), separated by sex.

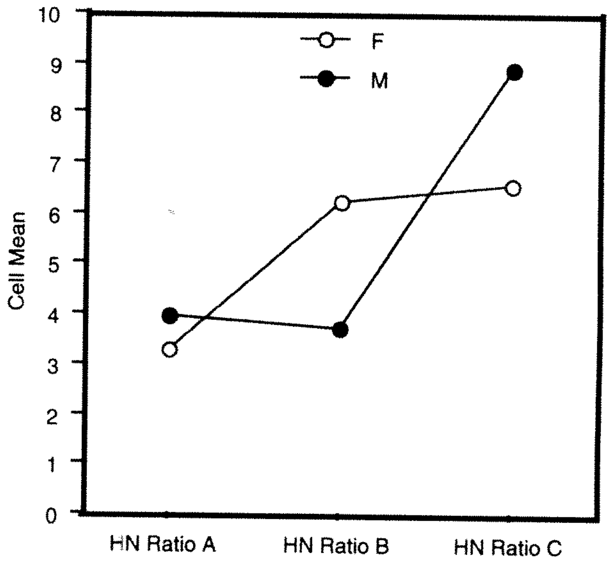

Fig 3. Mean (n = 35) harmonics-to-noise (HN) ratio before operation (A), after initial thyroplasty (B), and after revision thyroplasty (C), separated by sex.

Objective voice analysis (Table 3) was performed on representative 1-second audio samples before and after operation by means of the CSL 4300 software from Kay Elemetrics. Data samples were randomized and analyzed by both the surgeon and one observer. The results show that although subjective improvement was found in 80%, only one third to one half of these voices had improvement into the normal range for jitter and shimmer (33% and 47%, respectively), and the other half actually worsened. Mean raw data scores are shown in Figs 1, 2, and 3 for jitter, shimmer, and HNR, respectively, for both men and women. Correlation between video findings and subjective data showed no statistically significant patterns. However, glottic closure generally paralleled the voice analysis data for HNR (better glottic closure with increased HNR).

DISCUSSION

The cases presented confirm the long-held, but never proven, notion that the entire group of thyroplasty operations are revisable and, in some cases, reversible. The goal of the second operation was improvement of the result over the first operation,

TABLE 2. SUBJECTIVE VOICE PERCEPTIONS OF 35 PATIENTS

	Before Revision	*After Revision*
Improved; wants better voice	10	
Improved; satisfied with voice		28
Improved; subsequent worsening	10	2
Not improved	13	4
Worsened	2	1

TABLE 3. RESULTS OF REVISION THYROPLASTY

	Jitter	*Shimmer*	*Harmonics-to-Noise Ratio*	*Glottal Closure*
Men	(n = 15)	(n = 15)	(n = 14)	(n = 15)
Same	1	4	1	3
Better	7	6	10*	12
Worse	7	5	3	0
Women	(n = 16)	(n = 16)	(n = 16)	(n = 20)
Same	2	7	0	4
Better	10	5	10*	14
Worse	14	4	6	2

*Statistically significant.

and in every case, I was able to perform the revision without untoward difficulty. That is not to say that every patient was greatly improved symptomatically, for the voice is never totally normal after thyroplasty surgery. Minimum problems include loss of vocal volume when trying to shout, raise the voice, or sing in the higher range. In addition, the postoperative voice change may be compounded by initial traumatic scarring or the use of Teflon with a subsequent inflammatory response and granuloma formation. Finally, any preexisting disease (muscle wasting, neurologic, etc) will not be changed with repositioning of the vocal folds.

The most common reasons for failure in type I thyroplasty are window misplacement (too high posteriorly) and improper depth of medialization. Care is taken at the revision operation to draw the vocal fold line in its correct position, for everything else is based on this proper placement of the window. The use of the silicone block to hold the type I window cartilage in a depressed position has added greatly to the ease of revision. The silicone is inert, being completely covered by a fibrous sheath of scar approximately 1 mm thick after healing. Removing the block does not disturb the cartilage medialization, because of this fibrous growth. The internal scar, however, must be incised through to muscle to free the cartilage once again. Depending on the degree of dissection at the original operation and on postoperative healing, the ease of cartilage remobilization will vary greatly. However, in this series, all revisions of type I thyroplasties done with silicone, and 3 other reversals, were found to be technically possible, bringing the inner perichondrium of the window cartilage level with the inner perichondrium of the surrounding thyroid cartilage.

The midline type II thyroplasty is revised by removal of the silicone placed at the first operation and either widening or narrowing of the midline distance between the cartilage halves. The lateral type II has not been revised externally to date, and any further surgery needed for voice enhancement or airway improvement is done by direct laryngoscopy (eg, medial arytenoidectomy, vocal fold mucosal tightening, etc). Type IV thyroplasties are relatively easy to do, although it is still difficult, even with the patient awake, to choose the ideal amount of tension necessary to improve the voice.

Despite initial fears that the lateral approach to the arytenoid cartilage could only be performed once, I have found that it is feasible to do it more than once. If an initial arytenoid adduction has failed to significantly medialize the posterior glottis, the "revision" will usually require conversion to an arytenoid fixation to completely expose the cricoarytenoid joint and fix the arytenoid cartilage and its vocal process in a more favorable and medial position.

Both objective and subjective voice studies showed improvement after revision thyroplasty, with objective measurements being statistically significant for shimmer in female patients and HNR for all patients. Subjectively, 80% of patients were pleased and satisfied with their voice improvement, commenting that their voice was easier to produce, was more dependable when speaking, and caused them less vocal fatigue and neck muscle tension. If this improvement is added to improvements that occurred after just one operation, the total rate of improvement after thyroplasty is over 90%.

CONCLUSION

My experience with all thyroplasty operations has shown that it is possible to revise all types of thyroplasty operations, and that subjective improvement occurs in 80% of patients. In addition, vocal strength is increased (improved HNR), and vocal fatigue and instability are decreased. Thus, revision thyroplasty may significantly help a patient's voice if dysphonia persists after the initial thyroplasty.

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SAFETY AND EFFICACY OF TRANSCERVICAL RESECTION OF PARAPHARYNGEAL SPACE NEOPLASMS

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There are several surgical approaches for resection of parapharyngeal space (PPS) neoplasms. The purpose of this study was to evaluate local disease control, facial nerve injury, and need for mandibulotomy associated with resection of PPS neoplasms via the transcervical approach with submandibular gland excision. A retrospective chart review of 33 patients who underwent resection of a PPS neoplasm between October 1991 and July 2000 was performed. Of the 33 patients, 3 patients developed local recurrence after a median follow-up of 24 months. None of the patients experienced facial nerve paresis or paralysis. Three patients (9.1%) required a mandibulotomy for further exposure. This study demonstrated that the transcervical approach with submandibular gland excision for resection of PPS neoplasms provides excellent local disease control with minimal risk of facial nerve injury or need for mandibulotomy and/or tracheotomy.

KEY WORDS — parapharyngeal neoplasms, parapharyngeal space, transcervical excision.

INTRODUCTION

The parapharyngeal space (PPS) is a complex anatomic region located between the mandibular ramus and the lateral pharynx and extending as an inverted pyramid from the skull base superiorly to the hyoid bone inferiorly. Within this potential space are cranial nerves IX through XII, the sympathetic chain, the carotid artery, the jugular vein, lymph nodes, and fatty tissue. Although uncommon, a variety of benign and malignant neoplasms may arise from the structures within and adjacent to the PPS. Because of the PPS's anatomic complexity, location, and surrounding vital structures, resection of PPS neoplasms can prove challenging to the head and neck surgeon.

Multiple surgical approaches to tumors of the PPS have been described in the literature.[1-4] These methods range from simple transoral resection to more complex cervical-parotid approaches involving facial nerve dissection and possible mandibulotomy and tracheotomy. This report describes our experience with approaching both benign and malignant PPS neoplasms via a transcervical route with submandibular gland excision. The purpose of this study was to evaluate local disease control, facial nerve injury, and the need for mandibulotomy associated with this transcervical approach to PPS tumors.

METHODS AND MATERIALS

This study is based on a retrospective review of 34 patients who underwent resection of benign or malignant neoplasms involving the PPS by the senior authors (D.E.S. and A.A.) from October 1991 to July 2000 at The Ohio State University. One patient with PPS involvement by tumor extension from another head and neck site was excluded from the analysis. The medical records of the remaining 33 patients were retrospectively reviewed, and data were collected on the surgical approach, frequency of mandibulotomy and/or tracheotomy, tumor histopathology, surgical complications, facial nerve function, disease status, and length of follow-up. The main clinical and pathological characteristics of the study patients are shown in Tables 1 and 2. There were 12 male and 21 female patients with a mean age of 52.8 years (range, 36 to 76 years). The length of follow-up was calculated from the date of surgery to the date of the most recent surveillance examination and ranged from 1 to 107 months, with a median follow-up of 24 months.

RESULTS

The histopathologic diagnoses of the PPS lesions identified in this study are listed in Table 3. Twenty-four (73%) of the neoplasms were benign, and 9 (27%) were malignant. The most common neoplasm was pleomorphic adenoma (n = 16), followed by carotid and vagal paragangliomas (n = 4).

All patients underwent complete resection of clini-

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TABLE 1. PATIENT CHARACTERISTICS

Sex	
Male	12 (36%)
Female	21 (64%)
Age (y)	
Mean	53
Median	57
Range	36-76
Tracheotomy	
Yes	9 (27%)
No	24 (73%)
Mandibulotomy	
Yes	3 (9.1%)
No	30 (90.9%)
Disease status	
NED	27 (81.8%)
DOD	3 (9.1%)
AWD	3 (9.1%)

NED — no evidence of disease; DOD — dead of disease; AWD — alive with disease.

TABLE 2. PATHOLOGICAL DIAGNOSIS, DISEASE STATUS, AND LENGTH OF FOLLOW-UP

Patient	*Diagnosis*	*Disease Status*	*Follow-up (mo)*
1	Pleomorphic adenoma	NED	60
2	Chordoma	NED	69
3	Pleomorphic adenoma	NED	35
4*†	Carotid body paraganglioma	NED	41
5*	Carcinoma ex pleomorphic adenoma	AWD (distant)	26
6	Pleomorphic adenoma	NED	17
7*†	Vagal paraganglioma	NED	34
8	Fibromyxoma	NED	1
9*	Recurrent mucoepidermoid carcinoma	DOD (local)	1
10	Pleomorphic adenoma	NED	9
11	Papillary thyroid carcinoma	NED	24
12	Pleomorphic adenoma	NED	1
13	Pleomorphic adenoma	NED	27
14*	Squamous cell carcinoma	DOD (regional)	7
15	Skeletal muscle	NED	1
16	Pleomorphic adenoma	NED	24
17	Benign cyst	NED	25
18	High-grade mucoepidermoid carcinoma	NED	3
19	Spindle cell sarcoma	DOD (local)	14
20	Pleomorphic adenoma	NED	17
21	Hemangioma-lymphangioma	NED	1
22*	Pleomorphic adenoma	AWD (local)	47
23	Pleomorphic adenoma	NED	88
24	Vagal paraganglioma	NED	70
25	Vagal paraganglioma	NED	22
26	Pleomorphic adenoma	NED	29
27	Pleomorphic adenoma	NED	16
28	Pleomorphic adenoma	NED	8
29*†	Pleomorphic adenoma	NED	1
30*	Recurrent adenoid cystic carcinoma	NED	107
31	Pleomorphic adenoma	NED	2
32*	Neuroendocrine tumor	AWD (regional)	57
33	Pleomorphic adenoma	NED	32

*Tracheotomy performed.

†Mandibulotomy performed.

cally detectable disease by a transcervical approach with submandibular gland excision. In addition, a mandibulotomy was performed in 3 patients (9.1%) who were undergoing resection of carotid and vagal paragangliomas and a pleomorphic adenoma because of limited exposure of the superior extent of the disease.

Nine patients (27%) required tracheotomy either before or at the time of tumor resection. Tracheotomy was performed for definitive airway control in 3 patients who underwent mandibular osteotomy as part of their resection, and in 4 patients in whom extensive dissection along the pharyngeal wall was required, creating a concern for potential airway obstruction from pharyngeal edema. One patient required emergent tracheotomy for a hematoma following a transoral biopsy of a pleomorphic adenoma at another institution. In 1 patient with severe trismus, tracheotomy under local anesthesia was performed before definitive resection. Therefore, there were only 7 patients (21%) who required tracheotomy as a direct function of the surgical approach to tumor resection. Tracheotomy was performed in 5 of 9 (56%) and in 4 of 24 (17%) patients with malignant and benign tumors, respectively.

Disease status was evaluated in all patients to determine the effectiveness of the transcervical approach in controlling local disease. Twenty-seven patients (82%) had no evidence of disease after a median follow-up of 24 months. This group comprised 23 patients with benign neoplasms and 4 patients with malignant neoplasms.

Three patients died of disease. Two patients died of local disease from recurrent mucoepidermoid carcinoma and spindle cell sarcoma. The third patient, who was treated initially for an unknown primary squamous cell carcinoma involving the PPS, died of airway obstruction secondary to laryngeal cancer 7 months after resection of the PPS mass. In this patient, there was no evidence of disease locally involving the PPS.

Three patients were alive with disease. One patient developed distant metastases 26 months after resection of a carcinoma ex pleomorphic adenoma.

TABLE 3. HISTOPATHOLOGIC DIAGNOSES OF PARAPHARYNGEAL SPACE NEOPLASMS

Diagnosis	*Patients* *No.*	*%*
Benign	24	73
Pleomorphic adenoma	16	48
Paraganglioma	4	12
Hemangioma-lymphangioma	1	3
Fibromyxoma	1	3
Benign cyst	1	3
Skeletal muscle	1	3
Malignant	9	27
Mucoepidermoid carcinoma	2	6
Carcinoma ex pleomorphic adenoma	1	3
Chordoma	1	3
Spindle cell sarcoma	1	3
Adenoid cystic carcinoma	1	3
Metastatic disease	3	9

TABLE 4. POSTOPERATIVE COMPLICATIONS

Complication	*Patients* *No.*	*%*
Dysphagia	7	21
Velopalatal insufficiency	1	3
Trismus	4	12
Horner's syndrome	1	3
Cranial neuropathies	10	30
Nerve X	4	12
Nerve XII	6	18
Frey's syndrome	1	3

Another patient had local recurrence of a pleomorphic adenoma 43 months after surgery. She had undergone transoral excisional biopsy at another institution before definitive resection. The third patient developed distant and regional recurrences 30 months and 57 months, respectively, after resection of a PPS metastasis from a primary esthesioneuroblastoma that had previously been treated with surgery and postoperative radiotherapy.

Postoperative complications were identified in 14 of the 33 patients and are listed in Table 4. The most common complication, dysphagia, was identified in 7 patients. Six patients had hypoglossal nerve injury. Three cases of vocal cord paralysis occurred after resection of ipsilateral vagal paragangliomas, and 1 case after resection of metastatic esthesioneuroblastoma. There were no postoperative cases of permanent facial nerve paresis or paralysis. Resolution of postoperative complications occurred in 2 cases of dysphagia, 2 cases of trismus, 3 cases of hypoglossal nerve injury, 1 case of vocal cord paralysis, and 1 case of velopalatal insufficiency.

DISCUSSION

The transcervical technique used in this study involves incising the fascia of the submandibular gland inferiorly and reflecting the soft tissue away from the lateral surface of the gland so as to preserve the marginal mandibular branch of the facial nerve. The facial nerve is not visualized during the dissection. The submandibular gland is removed and the PPS is entered by means of blunt dissection to identify the neoplasm. Next, a combination of blunt and sharp dissection is used to remove the disease. If greater access is needed, the digastric, stylohyoid, and styloglossus muscles and stylomandibular ligament are divided. Occasionally, mandibular osteotomy is required for even more extensive exposure.

The histopathology of PPS neoplasms encompasses a variety of benign and malignant lesions. In this series, 73% of the lesions were benign and 27% were malignant; this distribution approximates those noted in larger studies.[5-7] Also in accordance with other studies, neoplasms of salivary gland origin were the most common, followed by neurogenic tumors. Among the 9 malignant PPS lesions, although mucoepidermoid carcinoma was the most common individual histopathologic diagnosis, metastatic lesions as a group were the most frequently encountered malignancy. Although the sample size is small, this finding differs from previous reports on PPS malignancies, in which adenoid cystic carcinoma was most common.[5,8]

One objective of this study was to evaluate local disease control of benign and malignant PPS neoplasms removed by the transcervical approach with submandibular gland excision. Three of the 33 patients (9.1%) in this study had persistent or locally recurrent disease over a median follow-up of 24 months (mean, 27.7 months). Two of these lesions were malignant (spindle cell sarcoma, mucoepidermoid carcinoma), and 1 was benign (pleomorphic adenoma). The first patient had a recurrence 43 months after resection of a pleomorphic adenoma. Before definitive resection, the patient had undergone transoral excisional biopsy at another institution. In addition, she required emergent tracheotomy for PPS and neck hematoma following her biopsy. Although frozen section margins were noted to be negative at the time of resection, small foci of disease were noted in areas of scar within the pharyngeal soft tissue corresponding to the previous biopsy site. She is currently alive with residual disease 47 months after her initial resection. The second patient had an initial diagnosis of a "blue cell tumor" on fine-needle aspiration. During operation, it was found that the disease had extensively eroded the skull base, with microscopically positive margins. The final diagnosis was a spindle cell sarcoma. Recurrence of gross disease was noted

2 months after the operation, and the patient was treated with chemoradiotherapy. Currently, he is alive with disease 11 months after his initial resection. The third patient underwent transcervical resection of a recurrent mucoepidermoid carcinoma involving the PPS 1 year after initial treatment with a total parotidectomy, partial maxillectomy, partial mandibulectomy, skull base resection, and intraoperative radiotherapy. Despite adequate exposure of the lesion, tumor was noted to be encasing the carotid artery at the skull base. She died of her disease 1 month after surgery.

In 3 additional patients, local disease control was obtained, but regional and distant metastases developed. One patient had an unknown primary squamous cell carcinoma of the PPS, but developed a primary laryngeal cancer and died of airway obstruction 7 months after resection of her PPS lesion. Another patient is alive with distant metastases from a carcinoma ex pleomorphic adenoma 26 months after PPS surgery. The third patient had a history of an esthesioneuroblastoma of the anterior skull base treated with surgery and postoperative radiotherapy that subsequently metastasized to the PPS. Thirty months after resection of the PPS lesion, the patient developed brain metastases that completely responded to chemotherapy. At 57 months of follow-up, he had regional neck disease that was to be treated with chemotherapy.

Information on disease control of PPS tumors in the literature is limited, probably because of the uncommon occurrence and the diversity of lesions involving the PPS. In his initial paper on the transcervical approach with submandibular gland excision for deep-lobe parotid tumors, Morfit[1] noted no evidence of tumor recurrence in 10 of 10 patients with mixed tumors and 1 of 2 patients with carcinoma with follow-up ranging from 8 months to 7 years. Another early study by Work and Hybels[8] reported 1 case of local recurrence in 33 benign tumors approached transcervically with facial nerve identification. Conversely, they noted that patients with malignancies of the PPS experienced a rapid downhill course leading to death. The sites of tumor recurrence in these patients were not specified. Allison et al[9] reported no evidence of recurrence in any of their 13 patients with pleomorphic adenoma involving the PPS after surgical resection by multiple approaches. Of their 4 patients with parotid malignancies, 1 died of local disease and 3 died of distant disease. More recently, Olsen[4] noted 1 local recurrence in a patient with adenoid cystic carcinoma and noted no local recurrences of benign tumors in his series of 44 patients with PPS lesions treated with a cervical-parotid approach. Hughes et al[5] reported on 172 cases of primary, previously untreated PPS neoplasms with a median follow-up of 6.1 years. The surgical approaches used were transparotid-cervical, transparotid, and transcervical. Of 137 patients with benign lesions, 12 patients (9%) developed local recurrence, while 27 of 35 patients (77%) with malignant neoplasms had recurrent or persistent local tumors. Twenty-four of these patients had local recurrence alone or in combination with regional and/or distant metastases.

The transcervical approach with submandibular excision provided excellent local disease control over the study period. This series includes previously treated, as well as primary, PPS lesions. Previous treatment may have contributed to an increased rate of local recurrence as a result of prior tumor spillage and seeding of surrounding tissue, increased difficulty in dissection, and difficulty in distinguishing scar tissue from recurrent disease. In the 2 cases with recurrence of malignancies, difficulty in local disease control did not appear to result from the surgical approach, but rather, from extensive skull base invasion and encasement of the internal carotid artery, which made meaningful oncological resection unattainable. Tumor recurrence in the patient with the pleomorphic adenoma was likely the result of tumor seeding from the prior transoral excisional biopsy, as histopathologic examination demonstrated small foci of tumor within the pharyngeal soft tissue. It is for this reason, as well as the risk of major neurovascular injury, that transoral biopsies or attempts at excision of PPS tumors are not recommended. Fine-needle aspiration biopsy is the preferred diagnostic method for tissue diagnosis, as it avoids the risk of seeding tumor and the possibility of major vessel injury.

In the remaining 23 patients with benign lesions, no evidence of disease recurrence has been noted during a median follow-up of 22 months (range, 1 to 88 months). The study period was 9 years, but several patients with benign tumors were lost to follow-up after only 1 postoperative visit, and they reduced the median follow-up. Ideally, longer follow-up in these patients would likely provide more definitive information on disease control, given the benign, slow-growing nature of many of these lesions.

Disease control was more difficult for patients with malignant PPS neoplasms. Of the 9 patients in this series with malignant neoplasms, 5 patients had disease recurrence. Two patients were alive with disease 26 months and 57 months after surgery, and 3 patients had died of their disease by 1 month, 7 months, and 14 months after surgery. The other 4 patients had no evidence of disease with follow-up periods of 3 months, 24 months, 69 months, and 107 months. Furthermore, all of these patients received adjuvant therapy with irradiation and/or chemotherapy.

An important concern in PPS surgery is the potential for injury to cranial nerves IX through XII and the sympathetic trunk due to the proximity to or involvement of these nerves with the local disease. With the exception of direct invasion by malignancy or a neurogenic tumor, permanent cranial nerve injury should be uncommon. In this series, dysphagia was the most common postoperative complication, occurring in 7 patients, and is likely a result of injury to the glossopharyngeal nerve. This complication was not disabling in any patient, and it resolved in 3 patients. Injury to the hypoglossal nerve occurred in 6 patients, with restoration of function in 3 patients. Three of these patients had PPS malignancies, 2 had paragangliomas, and 1 had prior surgical treatment involving the PPS.

Although not directly within the PPS, the facial nerve may also be at risk for injury, because of its proximity to this region. In an attempt to reduce the likelihood of facial nerve injury during tumor resection, some surgeons identify the main trunk and/or lower divisions of the facial nerve in PPS surgery, especially for deep-lobe parotid tumors.[4,8,10,11] With the transcervical approach, the main trunk and branches of the facial nerve are not identified. Care is taken to preserve the marginal mandibular branch by reflecting the fascia overlying the submandibular gland laterally away from the dissection. With this approach, no patient experienced permanent facial nerve paresis or paralysis. With meticulous dissection and tumor visualization, facial nerve injury can be avoided in virtually all cases without the need for routine nerve identification. Furthermore, potential injury incurred from facial nerve dissection can be prevented. Perhaps the use of facial nerve monitoring may provide an additional margin of safety with deep-lobe parotid tumors or those PPS lesions in close proximity to the stylomastoid foramen.

Although the transcervical approach provides direct access to the PPS and its associated neurovascular structures, some authors have expressed concern about limited exposure, need for finger dissection, and poor vascular control at the skull base with this approach.[3] This series shows no adverse results attributed to these concerns. If necessary, further exposure can be obtained by dividing the digastric, stylohyoid, and styloglossus muscles and stylomandibular ligament and retracting the mandible laterally. These maneuvers provide improved exposure and allow for both blunt and sharp dissection around the tumor while providing adequate vascular control. Mandibular osteotomy can provide additional tumor access, but is not commonly required. Midline mandibulotomy was necessary in only 3 patients (9.1%) with lesions involving the high PPS (2 paragangliomas, 1 pleomorphic adenoma). The incidence of mandibulotomy was comparable to or slightly lower than those reported in other series that involved various external approaches.[4,6,10] There were no complications associated with the mandibulotomy. However, a tracheotomy was required in these cases for airway protection.

As with mandibulotomy, the need for a tracheotomy with the transcervical approach is relatively infrequent. Tracheotomy was performed in 9 patients overall. In 7 patients, it was directly related to the surgical approach. Of those patients, tracheotomy was performed during operation for airway protection in 3 patients requiring mandibulotomy and in 4 patients after extensive dissection along the pharynx for resection of malignant neoplasms. Tracheotomy was required before the operation in the remaining 2 patients and was unrelated to the surgical approach. The first patient had severe trismus that precluded safe endotracheal intubation, and the other patient required emergent airway control because of a neck hematoma following transoral biopsy. Tracheotomy was performed more frequently in patients with malignant disease (5 patients) than in patients with benign disease (4 patients).

CONCLUSION

Multiple approaches have been described for the excision of PPS neoplasms. In this study, the transcervical approach with submandibular gland excision in a series of 33 patients provided satisfactory tumor exposure and vascular control without the need for mandibular osteotomy or tracheotomy in the majority of cases. Local disease control is excellent, but may be affected by previous treatment and histopathology. With good exposure and meticulous dissection, routine identification of the main trunk or lower division of the facial nerve is not necessary with this approach, and the risk of facial nerve injury is minimal. The transcervical approach provides a relatively safe and efficacious method for removing both benign and malignant PPS neoplasms.

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CELL BIOLOGY OF LARYNGEAL EPITHELIAL DEFENSES IN HEALTH AND DISEASE: PRELIMINARY STUDIES

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Esophageal epithelium has intrinsic antireflux defenses, including carbonic anhydrases (CAs I to IV) that appear to be protective against gastric reflux. This study aimed to investigate the expression and distribution of CA isoenzymes in laryngeal epithelium. Laryngeal biopsy specimens collected from the vocal fold and interarytenoid regions were analyzed by Western blotting and immunofluorescence. Carbonic anhydrases I and II were expressed by the majority of samples analyzed. In contrast, CA III was differentially expressed in the interarytenoid samples and was not detected in any vocal fold samples. The expression of CA III was increased in esophagitis as compared to normal esophageal tissue. Carbonic anhydrase I and III isoenzymes were distributed cytoplasmically in the basal and lower prickle cell layers. The laryngeal epithelium expresses some CA isoenzymes and has the potential to protect itself against laryngopharyngeal reflux. Laryngeal tissue may be more sensitive to injury due to reflux damage than the esophageal mucosa because of different responses of CA isoenzymes.

KEY WORDS — carbonic anhydrase, defense, gastroesophageal reflux, GERD, laryngeal epithelium, laryngopharyngeal reflux.

INTRODUCTION

Laryngopharyngeal reflux (LPR) differs significantly from classic gastroesophageal reflux disease (GERD). Patients with LPR have hoarseness, chronic throat clearing, globus, and cough, but do not commonly have heartburn.[1-3] Patients with GERD, on the other hand, have esophagitis and heartburn, but rarely have throat symptoms.[2] This is because the patterns and mechanisms of LPR and GERD are different.[4] Patients with LPR typically have upright (daytime) reflux with good esophageal function and no esophagitis, whereas GERD patients have supine (nocturnal) reflux.[1,4] Gastroesophageal reflux disease appears to be due to a dysfunction of the lower esophageal sphincter, while the primary defect in LPR appears to be upper esophageal sphincter dysfunction.[5] The mechanisms and manifestations of LPR and GERD are clearly different, but cannot be solely attributed to their reflux patterns. Some of the differences between LPR and GERD could also be due to the way in which these two epithelial tissues respond to exposure to gastric juice.

Laryngeal epithelium is probably more sensitive to injury by gastric reflux than the esophagus.[1,6] Experimentally, it has been shown that as few as 3 experimental reflux episodes per week can produce severe laryngeal damage.[1] The same is not true of the esophagus. In fact, in humans, on the basis of pH monitoring data, up to 50 reflux episodes a day (below pH 4.0) is considered normal. This type of physiologic reflux occurs mostly after meals.[1,7]

The larynx lacks significant extrinsic defenses. In the esophagus, peristalsis and salivary bicarbonate provide for restoration of neutral pH after a reflux episode has occurred.[8,9] Esophageal acid clearance is a combination of peristalsis (volume clearance of the acid bolus) and the subsequent delivery of salivary bicarbonate.[9] After an esophageal reflux event, the intraluminal pH remains low even after a swallow has cleared the acid bolus. Restoration of esophageal pH to neutral levels occurs as a result of the delivery of salivary bicarbonate by subsequent swallows.[8,9] The larynx has no comparable acid clearance mechanism. Other mechanisms may or may not be present to counteract a luminal gastric acid attack.

Among the most important intrinsic defenses of

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TABLE 1. CARBONIC ANHYDRASE ISOENZYMES

Isoenzyme	*Cellular Localization*	*Tissue Distribution*
I	Cytoplasmic	Erythrocytes,[14] esophagus,[17] gastric mucosa,[18] ileum and jejunum (weak expression),[19] large intestine and capillary endothelium,[19-22] eye,[23] pancreas[21]
II	Cytoplasmic	Erythrocytes,[14] gut epithelia,[17,19-22,24-26] pancreas,[15,16,21,24,27] liver,[21,28] gallbladder,[21] kidney,[24-26] eye,[23] skeletal muscle,[15] bronchial glands,[25] serous acinar cells of parotid and submandibular glands,[21,25,29] lung[15]
III	Cytoplasmic	Skeletal muscle,[15,24,30] erythrocytes,[14] esophageal epithelium,[17] kidney,[14] lung[14]
IV	Cytoplasmic	Lung,[28,31,32] kidney,[15,24,26,31] cardiac muscle,[28,33] esophagus,[17,21] small intestine,[19,22] epithelium and endothelium of large intestine,[19,22] skeletal muscle (rat),[28] liver,[16] eye,[34] endothelium of pancreas,[35] gallbladder[15]
VA	Mitochondrial	Gastric mucosa,[36] small and large intestine,[36] liver,[37-39] skeletal muscle[37,39]
VB	Mitochondrial	Pancreas,[38,40] kidney,[38,39] salivary glands,[38] spinal cord,[38] heart,[38] skeletal muscle,[38] liver [39]
VI	Secreted (glycoprotein)	Serous acinar cells of parotid and submandibular glands,[21,29,41] serum[41]
VII	Cytoplasmic	Messenger RNA expressed in cytoplasm of salivary glands,[15,16] brain (mouse)[42]
VIII*		Brain (mouse)[42]
IX	Membrane-associated	Pancreas,[35,43] gastric,[19,43] intestinal (excluding rectum),[19,43] and gallbladder mucosae,[19,43] cervical,[44] pancreatic,[35] colorectal,[45] and renal cell carcinomas[46]
XI*	Secreted[47]	
XII	Membrane-associated	Kidney,[48,49] salivary glands,[49] large intestine,[48,50] pancreas,[35,49] endometrium,[51] and renal cell,[48] colorectal,[50] and pancreatic carcinomas[35]
XIV	Membrane-associated	Kidney,[49,52] brain,[49,53] messenger RNA expressed in heart, skeletal muscle, lung, and liver,[49,52] colon,[49] small intestine,[49] urinary bladder,[49] spinal cord[49]

*Carbonic anhydrase–related protein. No known catalytic activity.

the esophageal mucosa are intracellular and secreted bicarbonates.[10,11] The ability of the esophageal mucosa itself to produce and secrete bicarbonate ions will augment the overall acid-neutralizing capacity of saliva. This ability of the esophageal mucosa provides an intrinsic protective mechanism by which the mucosa regulates the intracellular and extracellular pH during an acid load.[12,13] Previous research has not shown whether the larynx also possesses this capability.

Carbonic anhydrase (CA; EC 4.2.1.1) is a fundamentally important enzyme in the regulation of pH and in carbon dioxide transport. This zinc metalloenzyme is ubiquitously expressed in mammalian tissues. Its primary function is to catalyze the reversible hydration of carbon dioxide[14-16] in the reaction

$$\mathrm{CO2} + \mathrm{H2O} \overset{\mathrm{CA}}{\Leftrightarrow} \mathrm{HCO3}^- + \mathrm{H}^+$$

Currently, 11 catalytically active isoenzymes have been isolated and characterized, showing differences in activity, susceptibility to inhibitors, and tissue distribution. These constitute the mammalian CA multigene family. Table 1[14-53] summarizes the different distributions of CA isoenyzmes. Subcellularly, CAs I, II, III, and VII are expressed in the cytoplasm.[14-16,20-22] Carbonic anhydrases VA and VB are mitochondrial,[36-40,54] and CA IV is a membrane-associated glycoprotein.[28,31,55] Carbonic anhydrases IX, XII, and XIV, which were identified more recently, were found to be catalytically active transmembrane proteins, with their active sites located extracellularly.[19,43,48,49,52,53,56] Carbonic anhydrases IX and XII are receiving increased attention due to their overexpression in some tumors and may have a role as a potential marker for carcinogenesis.[35,44-46,50,56-60] As shown in Table 1, CA XI is a CA-related protein with no known catalytic activity; thus, CA VI is the only known secreted CA isoenzyme (active enzyme).[41,61,62]

The esophagus has been shown to express CA isoenzymes I to IV in the epithelium.[17] The isoenzyme distribution within the epithelium was related to enzyme activity, with the high-activity CAs II and IV localized in the suprabasal cell layers and the low-activity CAs I and III localized in the basal cell layers only. In an earlier study, the same authors[63] also demonstrated total CA activity in the submucosal glands, using the Hansson method.[64] Parkkila et al[21] also demonstrated a weak expression of CA II in the esophageal epithelium. In inflamed esophageal biopsy specimens, a substantial redistribution of CAs I and III to the prickle cell layer has been shown. Furthermore, CA II was also often expressed in the basal cell layer only, and CA IV was localized in the cytoplasm rather than on the membrane. These changes may represent an adaptive response to mucosal injury by gastric reflux.[65] Interestingly, total gastric CA activity has been shown to be reduced by bile salts and gastritis and is associated with a decrease in CA I expression.[18,66] Parkkila et al[67] have also demonstrated a reduction in salivary CA VI levels in patients with esophagitis and gastric and duodenal ulcers.

The defined roles for each CA isoenzyme in the esophagus remain speculative, but by acting in conjunction with ion exchange mechanisms, CA may provide mucosal protection in the form of bicarbonate ions. Thus, CA isoenzymes could aid in the neutralization of acid, consequently greatly reducing the activity of pepsins present. The importance of bicarbonate ions in this protection has been demonstrated by Tobey et al.[10] This hypothesis could also be applied to laryngeal defense against LPR.

The focus of these preliminary investigations was to determine whether laryngeal epithelium expresses CAs in a manner similar to the esophagus and whether responses to acid stimulation in the larynx (as in LPR) are similar to those in the esophagus.

Mucosal biopsy specimens were collected from 2 different laryngeal sites: the vocal fold and the interarytenoid region. The rationale for sampling both areas was the clinical observation that laryngeal neoplasms very rarely develop in the interarytenoid region. Laryngopharyngeal reflux is now receiving increased attention as a potential etiologic factor in the development of laryngeal cancer. Thus, we hypothesized that the interarytenoid epithelium might exhibit cellular protective mechanisms different from those of the vocal fold epithelium.

MATERIALS AND METHODS

Tissue Samples. Esophageal epithelial pinch biopsy specimens were collected from patients undergoing routine endoscopic examination at Ninewells Hospital and Medical School, Dundee, Scotland, as approved by the Tayside Medical Ethics Committee. Laryngeal epithelial biopsy specimens were collected from patients undergoing suspension microlaryngoscopy at Wake Forest University School of Medicine, Winston-Salem, North Carolina, as approved by the Institutional Review Board. All patients gave written informed consent. For both tissue types, specimens were collected in phosphate-buffered saline solution (PBS) on ice, snap-frozen in liquid nitrogen, and stored at –80°C for Western blot analysis. For immunofluorescent studies, specimens were fixed in 10% neutral buffered formalin (Sigma, St Louis, Missouri) and stored at room temperature until processed.

SDS-PAGE and Western Blot Analysis. Tissue extracts were prepared by homogenization in 50 μL of ice-cold lysis buffer followed by incubation at 0°C for 30 minutes. The lysis buffer consisted of 25 mmol/L Tris (pH 7.5), 7 mol/L urea, 50 mmol/L potassium chloride, 1 mmol/L phenylmethyl sulfonyl fluoride, 1 μg/mL leupeptin, 1 μg/mL pepstatin, 1 mmol/L ethylenediaminetetraacetic acid, 10 mmol/L sodium fluoride, 1 mmol/L dithiothreitol (DTT), 1 mmol/L benzamidine, 40 μg/mL bestatin, 100 μg/mL phosphoramidon, 10 μg/mL trans-epoxysuccinyl-L-leucylamido-(4-guanidino) butane (E-64), and 100 μg/mL *N*-tosyl-L-phenylalaninechloromethyl ketone (TPCK). The homogenates were then centrifuged at 13,000 rpm for 15 minutes at 4°C. The soluble supernatant was recovered for determination of protein concentration by the Bradford method.[68] All the reagents for sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) were from Bio-Rad Laboratories (Richmond, California) or Sigma, and electrophoresis was performed with a Bio-Rad Mini-Protean II electrophoresis cell. Aliquots of total protein (as indicated) were subjected to SDS-PAGE under reducing conditions according to Laemmli[69] in a 12% polyacrylamide gel. After electrophoresis, resolved proteins were transferred electrophoretically to Hybond-C nitrocellulose membrane (Amersham, UK) with a Bio-Rad Mini Trans-Blot transfer cell. Protein binding sites on the membranes were blocked with 5% (wt/vol) nonfat milk protein in 1% (vol/vol) Tween-20 in PBS (PBST) for 1 hour at room temperature. The membranes were then incubated for 1 hour at room temperature with antisera raised against human CA isoenzymes diluted 1:2,000 (sheep anti–CA I IgG, The Binding Site, Birmingham, England); 1:1,000 (sheep anti–CA II IgG, Serotec Ltd, Oxford, England); or 1:5,000 (murine monoclonal anti–CA III, Spectral Diagnostics, Toronto, Canada) in 1% PBST containing 5% nonfat milk protein. After being washed 3 times for 15 minutes in PBST buffer, membranes were incubated for a further 1 hour with either horseradish peroxidase–conjugated donkey anti-sheep IgG (SAPU Laboratories, Glasgow, Scotland) or horseradish peroxidase–conjugated rabbit anti-mouse IgG (DAKO Ltd, Cambridge, England) at a dilution of 1:1,500 in 1% PBST buffer containing 5% nonfat milk protein. After three 15-minute washings in PBST buffer, the polypeptide bands were detected by enhanced chemiluminescence (ECL) and visualized after exposure to Hyperfilm ECL.

The specificity of the antibodies for their respective isoenzymes was investigated by Western blot analysis. Briefly, 5 ng of human CA I and CA II purified proteins (both from Sigma) and 20 ng of human recombinant CA III protein (Spectral Diagnostics) were loaded onto a 12% SDS-PAGE gel. The proteins were subjected to electrophoresis and Western blotting as described above. The membranes were probed with anti-human CA I, CA II, or CA III antibodies (at dilutions detailed above), and the immunoreactivity was again visualized by ECL.

Immunofluorescent Detection of CA Isoenzymes.

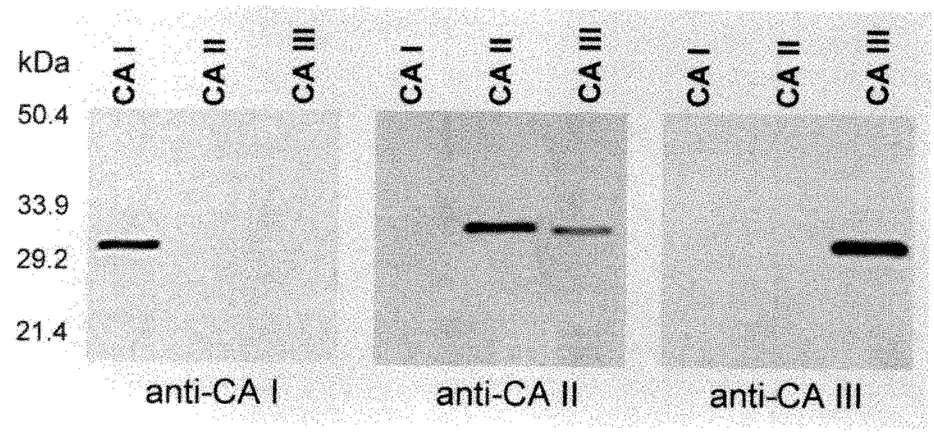

Fig 1. Specificity of polyclonal anti-human carbonic anhydrase (CA) I and II antibodies and monoclonal CA III antibody by Western blotting. CA I and II purified proteins (5 ng) and CA III recombinant protein (20 ng) were loaded onto 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis gels. After electrophoresis, proteins were visualized by immunoblotting with 1 of 3 anti-CA antibodies above. Results show that both anti–CA I and anti–CA III antibodies are highly specific for their respective isoenzymes, whereas anti–CA II shows weak cross-reactivity with CA III recombinant protein. All antibodies recognize major polypeptide bands at 30 kd.

Epithelial biopsy specimens were fixed with 10% neutral buffered formalin overnight at room temperature. The samples were dehydrated and then embedded in paraffin in a vacuum oven at 58°C, and sections (5 µm) were mounted onto Poly Prep poly-L-lysine–coated microscope slides. Nonspecific sites were blocked with normal serum for 30 minutes at room temperature in a humidified incubation chamber. The sections were then incubated overnight at 4°C with sheep anti-human CA I antibody or mouse anti–CA III antibody diluted 1/100 in 5% (vol/vol) normal serum in PBS. After incubation with primary antibody, the sections were washed twice in PBS for 10 minutes and then incubated for 1 hour at room temperature with fluorescein isothiocyanate (FITC)–conjugated donkey anti-sheep IgG or FITC-conjugated sheep anti-mouse IgG (both from SAPU Laboratories) diluted 1:100 in 5% normal serum in PBS. Excess antibody was removed by washing in PBS, and the sections were mounted onto coverslips with distrene tricresyl phosphate xylene. The tissue sections were examined with a Bio-Rad MRC 600 laser scanning confocal microscope.

Negative control sections were also prepared in the same way, except that the primary antibody was omitted in each case. Human esophageal epithelial sections previously shown to express CA I and CA III were used as a positive control tissue.[17]

RESULTS

Figure 1 shows that the anti–CA I and anti–CA III antibodies used here are highly specific for their respective isoenzymes. The polyclonal antisera against human CA II demonstrate a weak cross-reactivity with CA III. A single immunoreactive polypeptide band of 30 kd was detected in each case.

In normal esophageal tissue, CA III was found in the cytoplasm of basal and lower prickle epithelial cells (Fig 2A). The CA III expression became progressively weaker toward the luminal epithelial cells. Reactive cells displayed variable levels of immunopositivity. Inflamed esophageal tissues demonstrated increased expression of CA III as assessed by Western blotting (Table 2). Immunofluorescent staining of inflamed esophageal biopsy specimens revealed cytoplasmic localization of CA III in cells of the suprabasal layers with a greater number of immunopositive cells than in normal tissue (Fig 2B).

Immunoblotting of esophageal epithelial cell lysates demonstrated that CA III expression increased with malignant potential with a strong signal produced by samples of Barrett's epithelium and esophageal adenocarcinoma.

The expression of CA isoenzymes I, II, and III was also investigated in laryngeal mucosal biopsy samples from patients with documented LPR. The results are summarized in Tables 3-5. Carbonic anhydrase III was undetectable in all the vocal fold specimens analyzed and was differentially expressed in the interarytenoid samples. There was no detectable CA III protein in 44% (4/9) of the interarytenoid samples analyzed, while variable amounts were shown in the remaining 5 tissue samples. Carbonic anhydrase I was expressed by both tissues, with samples from the interarytenoid region displaying highly variable levels of expression compared with those from the vocal fold region. Carbonic anhydrase II was also detected in all vocal fold samples, but showed more variation in expression in the interarytenoid tissue extracts. The patient samples from both regions expressed at least 1 CA isoenzyme, and 2 samples from the interarytenoid region gave strong signals for all 3 isoenzymes. Figure 2C shows the localization of CA I protein in the vocal fold epithelium from

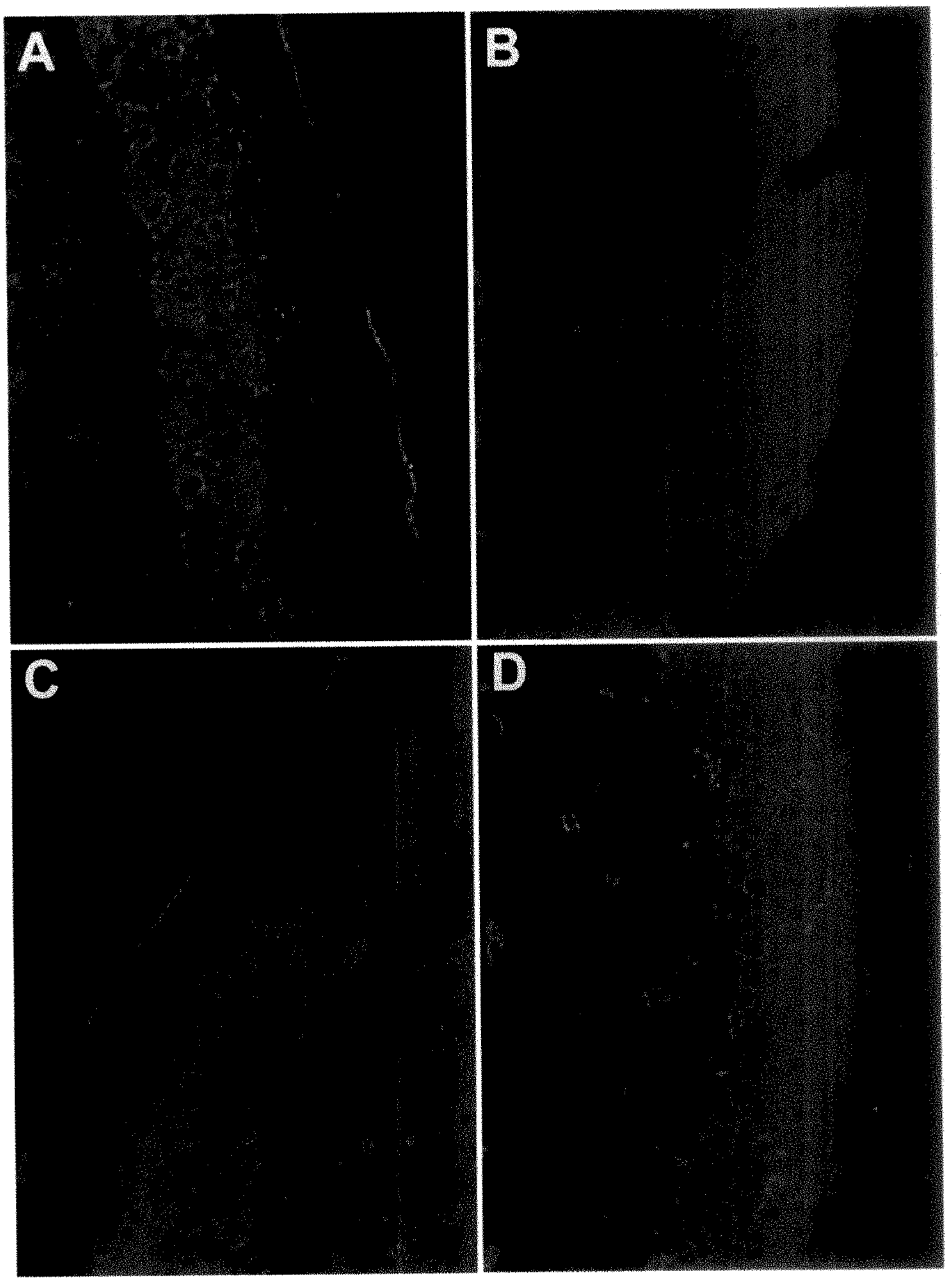

Fig 2. Immunofluorescent detection and localization of CAs in esophageal and laryngeal epithelia (original ×500). **A)** CA III in normal esophageal epithelium. Immunofluorescent staining of normal esophageal biopsy specimens reveals cytoplasmic localization of CA III in basal and lower prickle epithelial cells. **B)** CA III in esophagitis. In inflamed esophageal tissue, CA III is localized to cytoplasm of cells in suprabasal layers; ie, it appears to diffuse throughout epithelium, from basal layer to epithelial surface. **C)** CA I in vocal fold epithelium from patient with laryngopharyngeal reflux (LPR). CA I protein is found in cytoplasm of cells in basal and lower prickle layers. **D)** CA III in interarytenoid mucosa from patient with LPR. Immunopositivity is found in basal and lower prickle cell layers. CA III is present in cytoplasm of these cells.

a patient with LPR. Immunopositivity was found in the cytoplasm of the basal and lower prickle cell layers. Carbonic anhydrase III was also localized to the cytoplasm of the basal and lower prickle cells of the interarytenoid epithelium (Fig 2D).

TABLE 2. CARBONIC ANHYDRASE III IN HEALTHY AND DISEASED HUMAN ESOPHAGUS

	N	*Amount (Western Blot)*	*Location (Immunohistochemistry)*
Normal (healthy)	6	+	Basal and lower prickle cell layers
Esophagitis	3	++	Suprabasal layers
Barrett's esophagus	7	+++	Non-uniform distribution
Adenocarcinoma	1	+++	Not done

+ — weakly detected; ++ — moderately detected; +++ — strongly detected.

DISCUSSION

Table 6 summarizes the extrinsic and intrinsic antireflux defenses of the esophagus. The larynx has not been shown to have comparable acid clearance mechanisms. Saliva, for example, does not normally reach the larynx. Currently, information relating to the protective mechanisms of the larynx and the laryngeal response via those mechanisms to the presence of reflux is lacking. We have hypothesized that CA may be an important intrinsic defense mechanism in the laryngeal epithelium. In the esophagus, CA is potentially a key element of the antireflux barrier because of its ability to produce bicarbonate ions.[17] The pro-

TABLE 3. CARBONIC ANHYDRASE III IN LARYNGES OF LPR PATIENTS

	N	*Amount (Western Blot)*	*Location (Immunohistochemistry)*
Vocal fold tissue	7	ND	ND
Interarytenoid tissue	9	ND to +++	Basal and lower prickle cell layers

LPR — laryngopharyngeal reflux; ND — not detected; +++ — strongly detected.

TABLE 4. CARBONIC ANHYDRASE EXPRESSION LEVELS IN VOCAL FOLD TISSUE FROM PATIENTS WITH DOCUMENTED LPR

	Patient Number						
Isoenzyme	*1*	*2*	*3*	*4*	*5*	*6*	*7*
CA I	+++	++	+++	+++	+++	+++	+++
CA II	+++	+	+++	+++	++	++	++
CA III	ND	ND	ND	ND	ND	ND	ND

CA — carbonic anhydrase; ND — not detected; + — weakly detected; ++ — moderately detected; +++ — strongly detected.

tective properties of salivary and esophageal submucosal gland bicarbonate against acid have been demonstrated previously.[70,71]

In the present study we have demonstrated, by Western blot analysis, that the laryngeal epithelium expresses CA isoenzymes I, II, and III, albeit to variable extents. Surprisingly, the vocal fold epithelium did not demonstrate any detectable expression of CA III. The preliminary data reported on these 3 CA isoforms suggest that at least 1 of the intrinsic cellular antireflux defenses of the larynx may prove inadequate in some patients with LPR, since expression of only 2 isoenzymes was evident in many of the laryngeal samples tested. As compared to esophageal epithelium, which is known to express CA isoenzymes I to IV in normal and inflamed tissue,[17,65] it appears from these initial studies that the CA-derived buffering system of laryngeal epithelium might be more sensitive to the presence of gastric reflux. In contrast, esophagitis was associated with an increase in CA III expression, which is most likely to be a consequence of basal cell hyperplasia, a characteristic histopathologic sign of esophagitis.[72] Furthermore, Barrett's metaplasia and esophageal adenocarcinoma were associated with further increases in the levels of CA III protein. This finding may indicate that the CA III isoform conveys some properties conducive to malignant transformation, perhaps by limiting the reduction in intracellular pH and allowing the stress protein response to reduce apoptosis, consequently promoting cell survival in continuing acid.[73,74]

One further observation was that CA I and CA II levels often appeared to increase and decrease concurrently. For example, as seen in Table 5, interarytenoid tissue from patient 12 expressed low levels of CA I as compared with tissue from patient 13, and no detectable expression of CA II at all. Patients who do not demonstrate expression of either CA II or CA III, or express lower levels of these proteins, may have limited availability of intracellular and secreted bicarbonate ions and may therefore be more at risk of tissue injury by gastric reflux. In the absence of normal samples, we do not yet know whether the observed expression levels of CAs I, II, and III represent increases or decreases in protein levels in response to reflux-induced injury. For instance, CA III may in fact be overexpressed in the interarytenoid region during LPR, while not being expressed in the vocal fold epithelium. The vocal fold tissue may thus be more susceptible to injury. Confirmation would require sampling from normal laryngeal epithelial tissue.

The decision to study interarytenoid as well as vocal fold epithelium was based on several clinical observations. First, although interarytenoid mucosal hypertrophy is a known manifestation of LPR, posterior laryngeal complications of LPR are rare in the absence of concomitant endotracheal intubation. Second, primary squamous cell carcinoma of the larynx virtually never occurs in the interarytenoid region. In more than 20 years of practice, the senior laryngologist (J.A.K.) has encountered only 1 patient who developed carcinoma at this site, and that patient was a lifetime nonsmoker with severe LPR. Third, almost all nonsmokers who develop laryngeal carcinoma have documented LPR. And finally, the distribution of laryngeal papillomas in most cases spares the posterior larynx, with the exception of patients with LPR. On the basis of these observations, we postulated that the interarytenoid region may be less susceptible to reflux-related tissue injury than the vocal fold. Thus, we elected to investigate the cellular defenses of the

TABLE 5. CARBONIC ANHYDRASE EXPRESSION LEVELS IN INTERARYTENOID TISSUE FROM PATIENTS WITH DOCUMENTED LPR

	Patient Number								
Isoenzyme	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*
CA I	++	+++	+++	++	+	+++	+	+++	++
CA II	++	+++	++	ND	ND	++	+	+++	ND
CA III	ND	+++	+	++	ND	ND	ND	++	+++

ND — not detected; + — weakly detected; ++ — moderately detected; +++ — strongly detected.

TABLE 6. ESOPHAGEAL ANTIREFLUX DEFENSES

- Extrinsic defenses
  - Lower esophageal sphincter
  - Esophageal acid clearance
  - Peristalsis
  - Salivary bicarbonate
  - Salivary mucus
  - Salivary epidermal growth factor
- Intrinsic defenses
  - Intercellular bicarbonate
  - Epithelial and glandular carbonic anhydrase
  - Cell adhesion molecules and cell junctions
  - Subepithelial blood flow
  - Epidermal growth factor production by esophageal submucosal glands
  - Ion exchange mechanisms
    - Na^+-dependent, amiloride-sensitive Na^+/H^+ exchanger
    - Na^+-dependent Cl^-/HCO_3^- anion exchanger
  - Glycocalyx surrounding cell surface
  - Membrane coating granules releasing acidic and neutral mucosubstances
  - Heat shock proteins (stress proteins)
  - Epithelial proliferation and maturation

two sites, suspecting that they might be different, and that any differences might provide information relating to the pathophysiological changes that occur during LPR. We also hypothesized that defenses of the larynx against gastric reflux might be different from those of the esophagus.

The findings in patients with esophagitis suggest that expression of CA enzymes is modified. This may represent an adaptive response to mucosal injury and may contribute to increased secreted bicarbonate in the extracellular spaces, where acid will be present, as well as potentially enhancing the buffering capacity within cells. Using rabbit esophagus, Tobey et al[75] demonstrated a greater buffering capacity in prickle cells at acidic pH values than in basal epithelial cells, which would support the above hypothesis. The fact that CA isoenzymes in the larynx do not show the same pattern of response in the presence of LPR could indicate that the more luminal cells of the laryngeal epithelium may have a weaker resistance to acid during a reflux episode.

Further investigations are required to establish the pattern of expression and distribution of CA isoenzymes in normal laryngeal tissues not exposed to gastric reflux. This approach is currently limited by the difficulty of obtaining histologically normal epithelial tissue. Other factors, such as smoking, may influence CA expression in laryngeal tissues and warrant investigation, particularly since smoking is also a potent cause of reflux. In future studies, we aim to correlate the laryngeal CA findings in LPR patients with 1) clinical measures of LPR severity, 2) tissue inflammation scores, 3) mucin gene expression profiles,[76] and 4) heat shock proteins.[77,78]

An Na^+-dependent, amiloride-sensitive Na^+/H^+ exchanger has been demonstrated in rabbit esophageal prickle and basal cells that protected these cells from acid damage, most likely by the extrusion of H^+ ions.[75] The same authors also provided evidence of this cation exchanger in human esophageal cells.[79] Rabbit esophagus has also been shown to possess an Na^+-dependent Cl^-/HCO_3^- anion exchanger that is probably responsible for the secretion of bicarbonate ions into the extracellular space.[80] The presence and activity of ion exchange mechanisms in the larynx require further study and are likely to be the controlling factors in determining the bicarbonate-releasing potential of laryngeal epithelial cells in response to attack by luminal gastric refluxate.

CONCLUSIONS

The presence of CA isoenzymes I, II, and III in the laryngeal epithelium as a source of bicarbonate suggests an intrinsic mucosal defense mechanism that protects against tissue damage during LPR. The CA III isoenzyme was undetectable in vocal fold tissue. The absence of this CA isoenzyme may increase the susceptibility of the vocal fold tissue to reflux-related damage. Conversely, expression of CA III in the interarytenoid region may allow this tissue to be better protected from LPR. The increased expression of CA III in diseased esophageal epithelium suggests that laryngeal epithelium responds quite differently from esophageal tissue in response to gastric reflux.

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SURGERY FOR PEDIATRIC SUBGLOTTIC STENOSIS: DISEASE-SPECIFIC OUTCOMES

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To set the foundation to develop a disease-based, operation-specific model to predict the outcome of pediatric airway reconstruction surgery, we performed a retrospective database review of children operated on at a single, tertiary-care children's hospital. Over the 12-year period 1988 to 2000, a total of 1,296 airway reconstruction procedures were performed. Out of these, charts were identified for 199 children who underwent laryngotracheal reconstruction for a sole diagnosis of subglottic stenosis. Children were excluded from the study if their disorder included supraglottic, glottic, or upper tracheal disease. The main outcome measures were Myer-Cotton grade-specific decannulation and extubation rates, including both operation-specific and overall results. There were 101 children who underwent double-stage laryngotracheal reconstruction. The operation-specific decannulation rates for Myer-Cotton grades 2, 3, and 4 were 85% (18/21), 37% (23/61), and 50% (7/14) (χ^2 analysis, $p = .0007$). The overall decannulation rates were 95% (20/21), 74% (45/61), and 86% (12/14) (χ^2 analysis, $p = .04$). There were 98 children who underwent single-stage laryngotracheal reconstruction. The operation-specific extubation rates for Myer-Cotton grades 2, 3, and 4 were 82% (37/45), 79% (34/43), and 67% (2/3) (χ^2 analysis, $p = .63$). The overall extubation rates were 100% (45/45), 86% (37/43), and 100% (3/3) (χ^2 analysis, $p = .03$). Logistic regression analysis showed no effect of age (less than or greater than 2 years of age) on operation-specific or overall outcome parameters. We conclude that laryngotracheal reconstruction for pediatric subglottic stenosis remains a challenging set of procedures in which multiple operations may be required to achieve eventual extubation or decannulation. Children with Myer-Cotton grade 3 or 4 disease continue to represent a significant challenge, and refinements of techniques are being examined to address this subset of children. Disease-based, operation-specific outcome statistics are the first step in the development of a meaningful predictive model.

KEY WORDS — laryngotracheal reconstruction, outcome, subglottic stenosis.

INTRODUCTION

Surgical management of pediatric subglottic stenosis has undergone an evolution in the methods of diagnosis, workup, and treatment since Grahne[1] applied the Rethi procedure to children in 1971 and Fearon and Cotton[2] introduced the concept of cartilage augmentation and laryngotracheal reconstruction (LTR) in 1972. Since that point, there have been multiple series reporting surgical outcomes for the various operative procedures. Early work focused on the results of double-stage LTR; among these reports was the landmark work of Cotton et al[3] in which they noted an overall decannulation rate of 92% for 203 children who underwent airway reconstruction. More recent work documented the introduction and success of single-stage LTR[4-6] and of cricotracheal resection.[7,8]

Several different methods have been implemented to report the surgical outcomes for procedures designed to treat subglottic stenosis. Initially, articles reported the severity of stenosis according to the Cotton classification system[9] and reported outcomes on the basis of this scale.[3,10] Since 1994, the most common grading system used to assess subglottic stenosis has been that proposed by Myer et al,[11] and surgical outcomes studies have used this system to describe and define the different surgical populations.[8,12,13]

Surgical outcomes have also been described by reporting results of different surgical procedures without fully defining the disease population in which the surgery was done. The difficulty with interpreting "surgery-based" outcomes analyses such as these lies in the inherent differences between the different disorders. A posterior costal cartilage graft may well have a different outcome when used for isolated posterior glottic stenosis than when used for either posterior subglottic stenosis or for combined glottic and

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TABLE 1. OVERALL DEMOGRAPHIC DATA

	No. of Children	*Age (y; mean ± SD)*
Total	199	4.45 ± 5.09
Single-stage procedures	101	4.28 ± 4.46
Double-stage procedures	98	4.62 ± 5.67

Data on gender were missing for 4 children. There were 120 male patients and 75 female patients.

subglottic stenosis.

Other aspects of reporting on the outcomes of surgery for pediatric subglottic stenosis have included overall decannulation or extubation rates and the number of surgical procedures required to achieve this final goal.[3,10] While the number of procedures gives extremely important information to supplement the overall success rates for surgery, the reporting of "operation-specific" success rates (the rates at which an open surgical reconstruction is successful at achieving decannulation or extubation without subsequent open surgical repairs) is not currently part of the classic reporting paradigm. Such data would provide invaluable information to families who are contemplating surgery.

In this report we aim to use the most common classification system for pediatric subglottic stenosis[11] and to give disease-specific outcomes on a grade-by-grade basis. This method supports the use of "operation-specific" as well as "overall" outcomes. This report may provide a framework upon which future studies will flesh out more fully the model of "disease-based" analysis. It is clear that the presence or absence of tracheomalacia or gastroesophageal reflux disease may affect the surgical outcome; exactly how these conditions are defined needs clarification, and documentation to this point has been inexact. Future studies using this form of reporting may more fully characterize the disease process and the role of potential cofactors. We do not purport in this article to specifically compare particular surgical procedures (ie, cricotracheal resection versus anterior-posterior costal cartilage LTR). With these limitations in mind, we hope to establish a common means of presenting outcomes for pediatric airway reconstructive surgery by which results from different centers can be compared and analyzed.

MATERIALS AND METHODS

A retrospective database review was performed from a Microsoft Access database containing 1,296 pediatric airway reconstructions performed at the Children's Hospital Medical Center in Cincinnati (CHMCC) between the years 1988 and 2000. A pediatric airway reconstruction was defined as that performed in a child less than 18 years of age. The database included demographic information on each patient, as well as information regarding the type of surgery performed and the follow-up. Information regarding the date of decannulation for some patients was not available, and supplementary information was obtained from the patients' medical records and added to the database.

Patients were identified from this 12-year period who had isolated subglottic stenosis as their sole disorder. Patients with either concomitant supraglottic or glottic disease or with concomitant suprastomal collapse or tracheomalacia were excluded from the study. Patients whose surgical procedure consisted of an anterior cricoid split also were excluded from the study.

For purposes of uniformity, only those patients whose preoperative subglottic stenosis could be graded according to the Myer-Cotton grading system were included in the study; patients for whom this information was not available were excluded.

Patients who had been previously operated on elsewhere than at CHMCC were included in the study, as long as they met the other requirements listed above. Their initial Myer-Cotton classification was defined as that made when they were first seen at CHMCC.

With these inclusion and exclusion criteria, the charts of a total of 199 children were identified for data review. A Microsoft Access database was created to track demographic data, data on the surgical procedures performed, and outcomes data consisting of operation-specific extubation or decannulation rates and overall extubation or decannulation rates. "Operation-specific" decannulation or extubation rates are defined as the rates at which an *open* surgical reconstruction was successful at achieving decannulation or extubation without subsequent *open* surgical repairs. Endoscopic procedures such as dilation or use of the CO2 laser do *not* affect the operation-specific calculations.

RESULTS

For the 199 children identified, the average age at the time of operation was 4.42 years (SD, 5.09 years; Table 1). This figure includes the age at the time of the initial operation, as well as the age at subsequent operations. There was a male predominance of 1.6 to 1. The overall rate of decannulation or extubation was 84%.

There were a total of 297 procedures performed, for an average of 1.49 procedures per child (Table 2). Each child's airway was assessed before each reconstruction, and the relative grades of stenosis ob-

TABLE 2. OVERALL RESULTS

Number of procedures	297
Mean number of procedures per child	1.49
Overall decannulation or extubation rate	84% (165/199)
Degree of subglottic stenosis (Myer-Cotton grade) at time of surgery	
Grade 2	91 (31%)
Grade 3	157 (53%)
Grade 4	31 (10%)

served are listed in Table 2.

For purposes of data analysis, children were divided both according to their subglottic grade and according to whether they had a single- or a double-stage procedure. The type of procedure (LTR or cricotracheal resection) and the type of graft used were recorded for each of the populations (Table 3). The operation-specific and overall extubation rates for children with single-stage procedures and for children with double-stage procedures are shown in Table 4. The results for those children who underwent cricotracheal resections are reported in Table 5. Results for children with grade 1 stenosis are not included, because the numbers were too few.

A χ^2 analysis of each data set was performed to look for statistically significant differences. Logistic regression modeling was used to look for statistical differences as a result of age. Examination of the results of the χ^2 analysis for operation-specific and overall outcomes based on grade alone without accounting for age revealed that there was no statistically significant difference among Myer-Cotton grade 2, 3, and 4 stenoses in terms of operation-specific extubation rates for single-stage LTR ($p = .63$). There was, however, a statistically significant difference among the grades in terms of overall extubation rates for this population ($p = .03$). Analysis of the double-stage procedures revealed statistically significant differences among grades in both operation-specific decannulation rates ($p = .0007$) and overall decannulation rates ($p = .04$).

TABLE 3. ANALYSIS OF LARYNGOTRACHEAL RECONSTRUCTIONS

	Single-Stage	*Double-Stage*
Number of children	98	101
Number of procedures	132	162
Mean number of procedures per child	1.3	1.6
Number of children with		
Myer-Cotton grade 2 subglottic stenosis	45	21
Myer-Cotton grade 3 subglottic stenosis	43	61
Myer-Cotton grade 4 subglottic stenosis	3	14
Type of graft		
Costal cartilage	112	132
Thyroid cartilage	7	9
Septal cartilage		6
Auricular cartilage	4	4
Other (cricotracheal resection)	9	11

TABLE 4. RESULTS OF LARYNGOTRACHEAL RECONSTRUCTIONS

	Extubation Rates for Single-Stage Procedures		*Decannulation Rates for Double-Stage Procedures*	
Myer-Cotton Grade	*Operation-Specific*	*Overall*	*Operation-Specific*	*Overall*
2	82% (37/45)	100% (45/45)	85% (18/21)	95% (20/21)
3	79% (34/43)	86% (37/43)	37% (23/61)	74% (45/61)
4	67% (2/3)	100% (3/3)	50% (7/14)	86% (12/14)
χ^2 Analysis	$p = .63$	$p = .03$	$p = .0007$	$p = .04$

To evaluate the effect of age on LTR, we divided the population in 2 ways. The first grouping divided the children according to whether they were less than or more than 2 years of age. The second grouping divided the children according to whether they were less than 2 years of age, between 2 and 5 years of age, or more than 5 years of age. For both groupings, logistic regression analysis revealed no statistically significant differences in overall extubation rates for either the single-stage ($p = .47$) or the double-stage procedures ($p = .96$).

DISCUSSION

One of the central aims of reviewing large retrospective series of surgical outcomes is to be able to better predict the results of future operations. Such predictions are fraught with potential error because of the immense selection bias implicit in the choices of which charts or cases should be reviewed in this retrospective fashion. Selection biases may be involved in which patients are chosen for review or the outcomes parameters themselves that are chosen. The temptation is to choose those patients whose

TABLE 5. RESULTS OF CRICOTRACHEAL RESECTIONS (N = 20)

	Extubation Rates for Single-Stage Procedures		*Decannulation Rates for Double-Stage Procedures*	
Myer-Cotton Grade	*Operation-Specific*	*Overall*	*Operation-Specific*	*Overall*
2	0		100% (1/1)	
3	75% (6/8)	88% (7/8)	75% (6/8)	88% (7/8)
4	100% (1/1)		67% (4/6)	83% (5/6)

success rates are the highest. However, to provide meaningful information, both to compare between differing studies and to educate families whose children are faced with the need for airway reconstructive surgery, a clear and uniform reporting system would be invaluable. For example, outcomes for squamous cell carcinoma of the head and neck are reported according to disease-specific criteria by defining the "T (size), N (nodal status), and M (presence or absence of metastasis)" stage.[14,15] Such a system does not currently exist to define the disorders for which pediatric airway reconstructive surgery is required.

It is well known that results of surgical procedures vary according to the disorders the procedures aim to address. With reference to the field of airway reconstruction, it has been documented that the results of single-stage LTR vary according to whether tracheomalacia exists as a co-morbid factor along with subglottic stenosis.[5] Clearly defined "disease-specific" outcomes parameters are a first step in establishing a classification system as a predictive model for outcome. Combining "disease-specific" with "grade-specific" data according to well-established grading systems further facilitates the development of such a model. As stated above, this "disease-specific" model will require future modification to take potential co-morbid factors such as tracheomalacia and gastroesophageal reflux disease into account. This will be best accomplished by establishing the criteria to be examined and then performing prospective database collection. Our results represent an example of how such a model would allow for meaningful analysis of surgical outcomes.

The results described above fall within the ranges for surgical outcomes that have been previously reported.[3,5-8,10,12,13] It is important to note that the children seen at CHMCC often have undergone attempted repair of their airway disease before being seen at CHMCC; the surgical results therefore may not necessarily mirror the results of operating on a child's airway for the first time. Another important point to note is that as the time frame for charts examined extended until January 2000, several of the children were in the process of moving toward, but were not yet at, the point of decannulation; the success rates necessarily reflect this limitation. Nevertheless, several global comments can be made. It is reasonable that both the operation-specific and overall results for the single-stage procedures would be somewhat higher than those for the double-stage procedures. The very decision to perform a single-stage procedure implies that the surgeon believes that there is a significant chance of success and that a double-stage procedure is not essential. Another aspect worthy of mention is the overall results for grade 3 and 4 subglottic stenoses. This disease severity remains a difficult surgical dilemma; the results serve to reinforce why alternative means such as cricotracheal resection are being employed to treat these difficult cases. Exactly which procedures are best utilized according to the specific disorders remains to be elucidated.

It is of interest that in our series, age was not a statistically significant factor when children were operated on above and below the 2-year age mark. Although success with LTR before the age of 2 years has been reported,[10] the impact of age upon surgical outcome has not been previously evaluated in a large series. Similar to the comparison of single-stage versus double-stage procedures, the statistical comparison of outcomes regarding airway reconstruction according to age criteria may *not* reveal underlying selection bias in terms of those children who are chosen to be operated on at a younger age. Most commonly, only those children who, in the experience of the senior surgeon, have the greatest likelihood of surgical success will be either scheduled for surgery at a young age (less than 2 years of age) or scheduled for a single-stage procedure. Exactly what defines this delineation remains to be studied in a prospective analysis; as has been described above, variables would include the presence or absence of other co-morbid medical problems such as gastroesophageal reflux disease, tracheomalacia, and concomitant glottic disease. However, for select children, early airway reconstruction is clearly a feasible option.

Finally, the operation-specific extubation and decannulation rates seem at first glance to be remarkably low. However, when coupled with the overall success rates, the figures become more recognizable. This means of representing the data is similar to the more traditional means of reporting overall success rates and the average number of procedures used to achieve these figures. When faced with a family member who wants counsel as to what to expect as a result of an upcoming operation, the ability to predict the likelihood of success for that given procedure is important. It remains understood that each open surgical procedure will require several endoscopic evaluations and minor manipulations after the major procedure. Nevertheless, the ability to predict whether and how often subsequent major procedures will be required is a first step in the development of a disease-based, operation-specific predictive model.

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ESOPHAGEAL MOTOR FUNCTION IN LARYNGOPHARYNGEAL REFLUX IS SUPERIOR TO THAT IN CLASSIC GASTROESOPHAGEAL REFLUX DISEASE

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Laryngopharyngeal reflux (LPR) in otolaryngology patients appears to be different from classic gastroesophageal reflux disease (GERD). In particular, esophagitis and its principal symptom, heartburn, considered the diagnostic sine qua non of GERD, are often absent in LPR. It has therefore been postulated that LPR patients have superior esophageal function. Esophageal acid clearance (EAC) is a measure of the ability of the esophagus to restore neutral pH after reflux events have occurred. It is considered an excellent overall measure of esophageal function. The mean EAC can be calculated from 24-hour pH monitoring data. A comparison of EAC in patients with GERD and LPR has not been previously reported. To compare the EAC of 1) patients with LPR alone, 2) patients with GERD alone, 3) patients with both LPR and GERD, and 4) patients without either LPR or GERD, we studied 200 otolaryngological patients who had undergone 24-hour double-probe (simultaneous pharyngeal and distal esophageal) pH monitoring, 50 in each group. The subgrouping of each patient was determined by previously established pH monitoring criteria. We defined GERD as abnormal esophageal reflux and LPR as abnormal pharyngeal reflux. The patients with GERD had a mean (±SD) EAC of 1.44 ± 1.2 minutes, and those with LPR had a mean EAC of 1.00 ± 1.00 minutes ($p < .05$). The patients with both GERD and LPR had a mean EAC of 1.53 ± 1.01 minutes. The patients without reflux had a mean EAC of 0.53 ± 0.38 minutes. We conclude that patients with LPR have significantly better EAC than those with GERD. These data suggest that patients with LPR have superior esophageal function. This finding may clarify our understanding of the differences in mechanisms, symptoms, and incidence of esophagitis in patients with LPR and GERD.

KEY WORDS — esophageal acid clearance, esophagus, gastroesophageal reflux, laryngopharyngeal reflux, reflux.

INTRODUCTION

Laryngopharyngeal reflux (LPR), the backflow of stomach contents into the throat, also called extraesophageal reflux, is different from classic gastroesophageal reflux disease (GERD). In particular, fewer than 35% of patients with LPR have heartburn and/or esophagitis.[1-3] In addition, the patterns and mechanisms of reflux in LPR and GERD appear to be different. Patients with LPR have predominantly daytime, upright reflux, and GERD patients have predominantly supine, nocturnal reflux.[1-3] Because of these differences, it has been postulated that esophageal function in patients with LPR is superior to that in patients with GERD, who commonly demonstrate esophageal dysmotility.

Prolonged pH monitoring of the esophagus has been performed since the 1960s,[4,5] and it was subsequently popularized by DeMeester and Johnson.[6-8] It provided an ambulatory test to quantify gastroesophageal reflux (GER) that was an alternative to studies such as barium esophagography and endoscopy. The double-probe (simultaneous esophageal and pharyngeal) pH monitoring technique was first reported in 1986.[9] Although double-probe pH data have been reported in patients with LPR,[1,3,9] specific esophageal acid clearance (EAC) data have not been previously reported.

Esophageal acid clearance represents an important esophageal defense mechanism, and the mean EAC is an excellent measure of esophageal function. The EAC actually measures 2 components of esophageal antireflux defense.[1,10] The first is volume clearance. After a reflux episode has occurred, a subsequent swallow must clear the volume of the refluxate, but the intraluminal pH remains low. Next, with subsequent swallows, salivary bicarbonate is delivered, restoring the intraluminal pH to normal. Helm[10] experimentally demonstrated the mechanisms of EAC. After a 15-mL bolus of acid was placed in the esophagus, subjects were instructed to swallow every 30 seconds. After the first swallow, it could be seen that the intraluminal pH remained very low, and then with subsequent swallows, the pH was restored to neutral in a stepwise fashion. Thus, EAC requires good peri-

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CENTER FOR VOICE DISORDERS PARAMETERS FOR AMBULATORY DOUBLE-PROBE PH MONITORING

	Normal Values
Percent of time pH < 4 upright	<8.1%
Percent of time pH < 4 supine	<2.9%
Percent of time pH < 4 total	<5.5%
Total No. of esophageal episodes of pH < 4	<51
No. of pharyngeal episodes of pH < 4	0

staltic (motor) esophageal function, as well as saliva that naturally contains bicarbonate. As a corollary, Rosman et al[11] demonstrated that patients with xerostomia virtually always have abnormal EAC.

The calculated EAC can be determined by analysis of pH monitoring data. For example, consider that a patient has 100 minutes during a pH study in which the pH in the esophageal probe is less than 4.0, and consider that the patient has 100 reflux episodes during the study. By dividing the total time (pH < 4.0) by the total number of reflux episodes, one would then get the mean EAC. In this example, the mean calculated EAC would be 1 minute (100 minutes divided by 100 episodes).

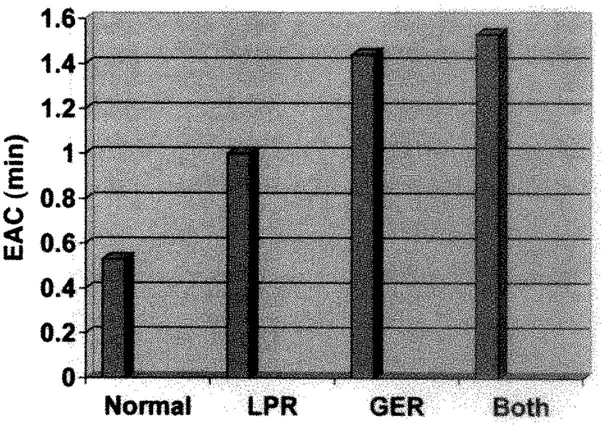
The purpose of this study was to use the pH-derived mean EAC as a measure of esophageal function in patients undergoing reflux testing for presumed LPR and/or GERD. The study was performed retrospectively, and on the basis of the results of each pH test, patients were assigned to 1 of 4 groups: 1) patients with LPR only, 2) patients with GERD only, 3) patients with both GERD and LPR, and 4) patients with normal pH study results (ie, normal esophageal and pharyngeal probe data). The hypothesis of this study was that patients with LPR alone would have significantly better (faster) EAC times than those with GERD.

MATERIALS AND METHODS

The data were extracted from the pH study database at the Center for Voice Disorders of Wake Forest University. Two hundred unselected otolaryngological patients with a clinical diagnosis of LPR who underwent ambulatory 24-hour double-probe pH monitoring formed the basis for this study.

In terms of pH monitoring technique, the distal pH probe was placed 5 cm above the lower esophageal sphincter, and the proximal probe was placed just above the upper esophageal sphincter, just behind the laryngeal inlet, by means of manometry. The technique has been previously described.[1,9,12-14] During each pH study, the percentage of time that the pH was less than 4.0 at both probes in both the upright and supine positions was recorded.

The parameters for the diagnosis of GER and LPR



Esophageal acid clearance (EAC; minutes) versus reflux status. LPR — laryngopharyngeal reflux, GER — gastroesophageal reflux.

(also previously reported[1,12,15]) are shown in the Table. These parameters for GER are standardized normative data from our center and are consistent with values used for the diagnosis of GER in most laboratories. We consider any pharyngeal acid exposure below pH 4.0 to be abnormal. We acknowledge that this criterion is controversial, but we believe that it is valid in patients with symptoms and physical findings consistent with LPR, such as those in this series.

On the basis of the 24-hour dual pH-probe data, 50 consecutive patients were selected to make up each of the study subgroups (N = 200). The study subjects were assigned to 1 of 4 groups: 1) those with GER alone, ie, abnormal esophageal reflux by pH criteria, but no pharyngeal reflux; 2) those with LPR alone, ie, abnormal pharyngeal reflux by pH criteria, but normal esophageal pH data; 3) those with both GER and LPR, ie, abnormal esophageal and pharyngeal acid exposure; and 4) those who had normal (esophageal and pharyngeal) pH study results.

The EAC for each subject was determined by dividing the total esophageal acid exposure time (minutes) when the pH was below 4.0 by the number of reflux events.[16] In addition, each study was examined to determine the pattern of reflux, ie, abnormal upright (daytime) reflux, abnormal supine (nocturnal) reflux, or both. A 1-way analysis of variance was used to evaluate the global association between EAC and reflux status. The independent-sample *t*-test was used to evaluate statistical differences in patient demographics and the mean EAC between individual study groups.

RESULTS

The 4 study groups did not differ significantly with regard to age or gender. The mean age of the entire cohort (N = 200) was 51 years, and 55% were female. The mean (±SD) EAC of patients with GER alone (1.44 ± 1.20 minutes) was significantly longer than the mean for those with LPR alone (1.00 ± 1.00 minutes; $p < .05$). Furthermore, patients with GER were 3.2 times more likely than patients with only LPR to have an EAC of more than 1 minute (95% confidence interval, 1.4 to 7.2). The mean EAC of those with LPR alone was also significantly longer than that of the normals ($p = .002$). The mean EAC for those with normal results was 0.53 ± 0.38 minutes. Between-group comparison revealed that patients with both GER and LPR had no significant difference in EAC from the GER group (mean EAC, 1.44 ± 1.2 minutes). The EAC data for all groups are shown in the Figure.

The pattern of reflux was also evaluated. For those with GER, abnormal supine reflux was present in 52%, but in the LPR-only group, supine reflux was seen in only 24%. Patients with LPR were 1.6 times more likely to have reflux exclusively in the upright position.

DISCUSSION

Esophageal acid clearance is an important mechanism that protects the esophageal mucosa from damage, ie, reflux esophagitis. The EAC reflects the overall function of the body of the esophagus by indirectly measuring overall esophageal peristaltic function. Although xerostomia was not measured in any of the study patients, we presume that all were normal with regard to salivary function; ie, all subgroups had similar levels of salivary bicarbonate. Thus, the data of this study strongly suggest that esophageal motor function is not as severely affected in LPR as in GERD.

That LPR patients have predominantly upright (daytime) reflux also appears to have significance. Patients with exclusively upright reflux would be presumed to have less esophagitis than those with supine reflux, because the total acid exposure times in the former group are less than those in patients with nighttime reflux and GERD.[1,3,9,12]

In summary, it appears that the reflux patterns and mechanisms of LPR and GERD differ: 1) LPR patients usually do not have heartburn or esophagitis; 2) LPR patients usually have upright (daytime) reflux (with supine reflux being most common in GERD); and 3) esophageal function in LPR is superior to that seen in GERD. The EAC data reported herein support these differences.

CONCLUSION

The EAC of patients with LPR is significantly better (faster) than that of patients with GER. This finding suggests that the underlying mechanisms responsible for GERD and LPR differ. Patients with LPR appear to have predominantly upright (daytime) reflux and good esophageal motor function, in contrast to GERD patients, who usually have supine nocturnal reflux and worse esophageal motor function. These differences help explain why LPR patients usually do not have esophagitis or heartburn.

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EFFECTS OF GUM CHEWING ON PHARYNGEAL AND ESOPHAGEAL PH

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We investigated the effects of gum chewing on pharyngeal and esophageal pH levels in patients with laryngopharyngeal reflux (LPR) who were undergoing reflux testing. Forty consecutive, unselected, adult patients who were undergoing ambulatory double-probe (simultaneous pharyngeal and esophageal) pH monitoring for diagnosis of LPR were asked to chew 2 sticks of gum 4 times during their pH studies. Twenty subjects chewed regular sugarless gum, and 20 subjects chewed a sugarless gum containing bicarbonate. The subjects recorded the beginning and end of each gum-chewing period. The mean pH values for the gum-chewing intervals and for comparable pre–gum-chewing intervals were analyzed statistically for both the pharyngeal and esophageal probe data. The regular gum group and the bicarbonate gum group were analyzed separately. In addition, the gum-chewing pH data were compared to controls, ie, normal postcibal buffering effects. The data show that gum chewing consistently increases esophageal and pharyngeal pH, and that bicarbonate gum causes greater increases than regular gum. For patients with LPR, gum chewing appears to be a useful adjunctive antireflux therapy.

KEY WORDS — bicarbonate, chewing gum, esophagus, gastroesophageal reflux, GERD, laryngopharyngeal reflux, pH monitoring, reflux, reflux treatment.

INTRODUCTION

Laryngopharyngeal reflux (LPR) is distinctly different from classic gastroesophageal reflux disease (GERD).[1] The hallmark of GERD is esophagitis and its principal symptom, heartburn. Patients with LPR have dysphonia, chronic throat clearing, globus, and dysphagia as the primary symptoms, and usually do not have heartburn (or esophagitis).[1] The differences between LPR and GERD may be explained in part by differences in the patterns and mechanisms of reflux in each group.[2] Typically, GERD patients have supine (nocturnal) reflux (with long periods of acid and peptic exposure in the esophagus). By comparison, LPR patients usually have daytime (upright) reflux with normal esophageal function and minimal nighttime reflux.[1,2] A certain amount of reflux is normal during esophageal pH studies, but so-called "physiologic reflux" in the pharynx is never considered normal.[1,2] In many LPR patients, postcibal symptoms persist despite aggressive antireflux treatment.

Unlike the esophagus, which secretes bicarbonate,[3] the laryngopharyngeal mucosa lacks this intrinsic protection against the effects of reflux. The larynx also lacks significant extrinsic sources of protection. In the esophagus, peristalsis and salivary bicarbonate provide for restoration of neutral pH after a reflux episode has occurred.[4] The laryngopharynx has no such acid clearance mechanism.

Gum chewing has been shown to increase salivary flow rates, salivary bicarbonate concentration, and the rate of swallowing.[5-7] In addition, increases in salivary flow appear to be a function of gum-stick size, ie, the larger the bolus of gum, the greater the increase in salivary flow.[7,8] Also, the specific beneficial effects of sugar-free gum on the mouth and esophagus have been reported.[5-8] We investigated the effects of gum chewing on esophageal and pharyngeal pH in patients who were undergoing ambulatory 24-hour double-probe (simultaneous esophageal and pharyngeal) pH monitoring.

MATERIALS AND METHODS

Forty consecutive, unselected adult patients who were undergoing ambulatory double-probe pH monitoring for evaluation of LPR were studied prospectively. The subjects were either not on antireflux therapy, or had been asked to stop therapy 1 week before testing, as is our routine for reflux testing. The protocol for this study was approved by the Wake Forest University School of Medicine institutional review board for human research.

The first 20 subjects were given 8 sticks of Wrigley's Winterfresh Sugarfree Gum and instructed to chew 2 sticks at a time. The subjects were instructed to chew for a minimum of 10 minutes and allowed to chew as long as they wished, and they were instructed to use an event marker to indicate the gum-chewing periods. Similarly, the second 20 patients were given 8 sticks of Arm and Hammer Sugarfree

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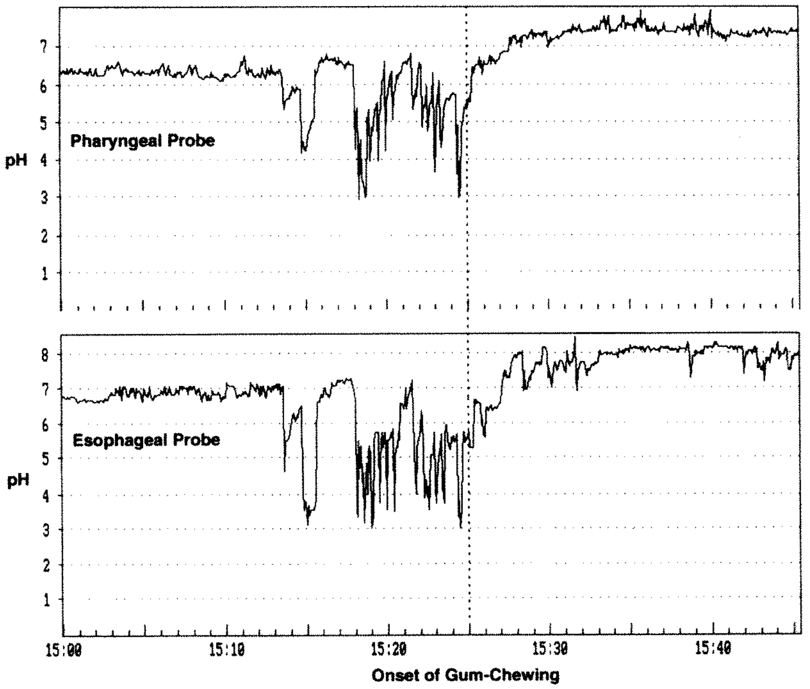

pH tracing demonstrates effects of gum chewing. Shown here is portion of double-probe (simultaneous esophageal and pharyngeal) pH tracing of study subject. Before gum chewing, subject demonstrated multiple esophageal and pharyngeal reflux episodes (to below pH 4), and he was symptomatic. Shortly after he began to chew bicarbonate gum, pH was restored to neutral (normal) in both esophagus and pharynx. In instance shown, disappearance of patient's symptoms coincided with gum chewing and with observed pH shifts.

Baking Soda Gum.

After completion of each study, the data were analyzed. The mean pH of each gum-chewing period was compared to that of the comparable time period just prior to gum chewing. The first 5 postcibal minutes were excluded, as it has been demonstrated that physiologic clearing of gastric contents may occur during that time. The data were analyzed by Student's *t*-test for both the pharyngeal and esophageal probes for both types of chewing gum. Similar statistical analysis was applied to the differences between the effects of gum chewing and control data.

For a long time, frequent small meals have been recommended for patients with reflux. Presumably, this recommendation was based upon the observation that small meals are less likely to cause reflux, but equally important, the antireflux effects of eating also are presumed to be related to the buffering effects of food on the stomach contents. Thus, the question that we attempted to address using controls was, Does food provide the same degree of buffering and postcibal symptom relief as does gum chewing?

Ten (other) patients with clinical LPR who were undergoing pH monitoring were used as the controls. The pH recordings of the control subjects were analyzed to determine the mean duration of the buffering effect of food (after completing a meal); ie, we measured the time from the end of each meal to the first postcibal reflux episode.

RESULTS

The regular gum (RG) and the bicarbonate gum (BG) groups were analyzed separately. In 92% (37/40) of the subjects, the mean esophageal and pharyngeal pH increased with gum chewing. For the RG group, the mean pharyngeal pH increased from 6.85 to 7.30 during the gum-chewing period ($p < .0001$), and the mean esophageal pH increased from 6.45 to 7.14 ($p < .0001$). For the BG group, the mean pharyngeal pH increased from 6.70 to 7.43 during gum chewing ($p < .0001$), and the mean esophageal pH increased from 6.44 to 7.49 ($p < .0001$). In some subjects, gum chewing appeared to completely abolish reflux events (see Figure).

The mean (±SD) time of gum-chewing episodes was 25.2 ± 16.0 minutes, and the mean buffering effect of BG chewing was 49.1 ± 23.6 minutes. This difference was highly significant at the $p < .0001$ level. On average, the beneficial effect of gum chewing on pH was maintained 2.4 ± 1.3 times as long as the gum-chewing period (range, 1.1 to 7.0 times), and in no case did reflux occur during gum chewing.

The control data revealed that the mean buffering effect of meals — the time from the end of the meal to the first postcibal reflux episode — was 27.4 ± 18.2 minutes. The difference between the mean duration of the gum-chewing effect and the mean duration of the postcibal buffering effect (controls) was also statistically significant ($p = .002$).

DISCUSSION

On the basis of anecdotal reports of LPR patients who experienced relief of LPR symptoms (particularly postcibal symptoms) after gum chewing, a literature review was performed. It revealed that significant increases in salivary flow and salivary bicarbonate concentration are associated with gum chewing, and that chewing gum appears to be therapeutic for GERD.[6-8] The study herein reported is the first aimed at assessing the potential benefits of gum chewing on reflux in LPR patients.

Esophageal acid clearance is a combination of peristalsis (volume clearance of the acid bolus) and the subsequent delivery of salivary bicarbonate.[4] After an esophageal reflux event, even after the acid bolus has been cleared (by a swallow), the intraluminal pH remains low. Restoration of esophageal pH to neutral levels occurs as a result of the delivery of salivary bicarbonate by subsequent swallows. Although gum chewing may also increase swallowing rates and peristalsis, this hypothesis has not been studied.

Our data indicate that substantial and significant pH shifts follow gum chewing in most subjects. On average, the beneficial (buffering) effects of chewing BG last more than twice as long as the actual gum-chewing periods, and the beneficial effects appear to be significantly longer than the buffering effects of ordinary meals (49 minutes versus 27 minutes; $p = .002$). These data are consistent with our clinical experience that BG gum chewing can provide dramatic symptomatic relief for LPR patients, especially those who have symptoms after meals.

We currently recommend that patients with postcibal symptoms chew 2 pieces of BG one half-hour after a meal or at the time of onset of symptoms, whichever comes first. In addition, we recommend gum chewing for LPR patients at certain other times, depending on symptom patterns, eg, before (or after) exercise, before going to bed, etc. Although the effect is difficult to quantify, chewing BG appears to be an inexpensive, well-tolerated, and effective adjunct to traditional drug therapy for LPR.

CONCLUSIONS

Our data demonstrate that gum chewing consistently increases both esophageal and pharyngeal pH, and that in LPR patients, BG chewing appears to be a useful adjunctive antireflux therapy.

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COMPARISON OF THE PHONATION-RELATED STRUCTURES AMONG PIG, DOG, WHITE-TAILED DEER, AND HUMAN LARYNGES

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There is an important need for good animal models of the larynx for the study of the physiology of phonation. The dog's larynx has been used as an animal model for more than 2 centuries of phonatory research. However, there is some evidence that the pig larynx has advantages over the dog larynx as a model of phonation. Another larynx that is readily available is the deer larynx. In this comparative study, the laryngeal anatomy and function were examined in 4 species — human, pig, dog, and white-tailed deer. Particular attention was directed to those structures that one would predict could affect phonation, from the anatomic and biomechanical point of view. Although the vocal fold length was similar for all 4 species, the larynges described differed in some phonation-related characteristics. The data suggest that from a structural perspective, the pig larynx is a superior model for phonatory research.

KEY WORDS — comparison, deer, dog, human, larynx, pig, structure.

INTRODUCTION

Since fresh human larynges are difficult to obtain, good animal models of the larynx are essential aids in the study of phonatory laryngeal function. The canine larynx has been used for more than 2 centuries of phonatory research.[1] Canine and human larynges possess physical similarities that allow the dog larynx to be used as a model for studying mechanisms of human phonation.[2-6]

However, there is some evidence that the pig larynx can be useful as a phonation model. A comparative study of vocal fold structure[7] demonstrated that the thickness of the mucosa of the dog vocal fold is about 3.0 mm, ie, 3 times thicker than the typical vocal fold mucosa in humans (1.1 mm). The thickness of the pig vocal fold mucosa (0.9 mm) is similar to that in humans. Moreover, the ratio of the length of the vocal fold membranous portion to the thickness of the mucosa is 20:1 in pigs, 14:1 in humans, and 5:1 in dogs. The same study suggested that the distribution and density of elastic and collagenous fibers of the pig larynx more closely resemble those of humans than those of dogs. Because the deep portion of the lamina propria of a dog is less dense and more pliable, the mucosal wave in dogs demonstrates much greater wave amplitude than that in humans.[8] In addition, concerns about the use of domestic animals for research have greatly decreased the availability of dogs and the desirability of using the canine model. Pig larynges are readily available from slaughterhouses without concerns that the animals are being sacrificed for research purposes.

Another larynx that is readily available is the deer larynx. Its gross appearance and size are similar to those of human and dog larynges. However, little is known about the deer larynx as a phonation model.

This comparative study examined the laryngeal anatomy and function of 4 species: human, pig, dog, and white-tailed deer. Attention was focused on anatomic and biomechanical features critical to phonation.

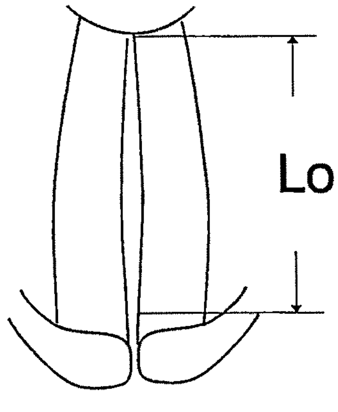

Fig 1. Method of measuring length of membranous vocal fold (Lo).

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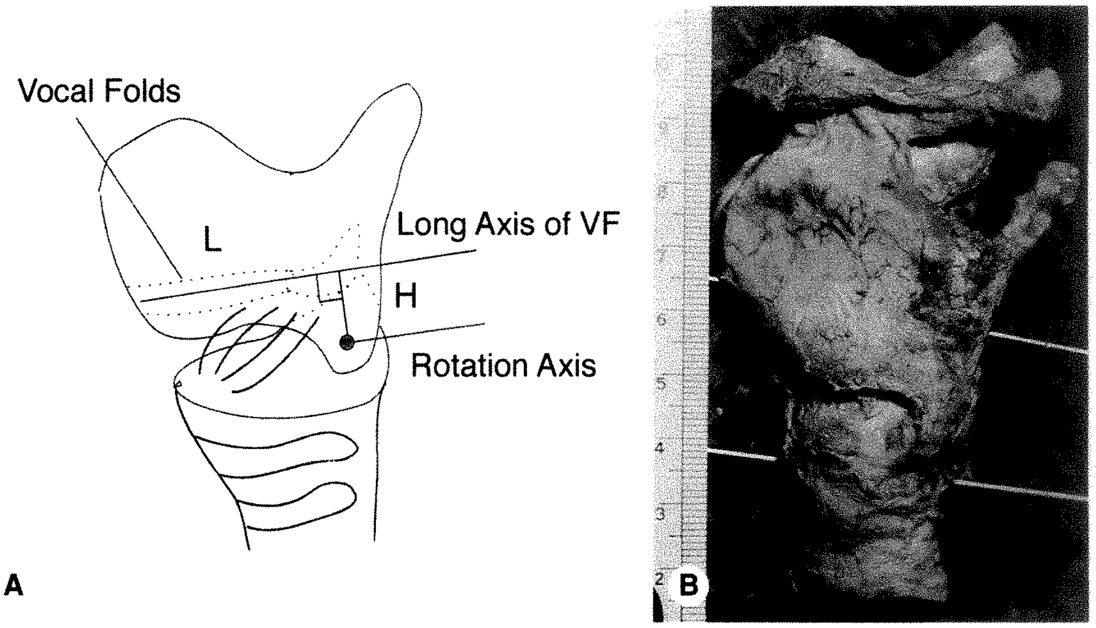

Fig 2. Vocal fold (VF) height. **A)** Distance from axis of rotation of cricothyroid joint to longitudinal axis of VF was defined as height of VF (H). L — length of membranous VF. **B)** Long needle was inserted parallel to VF body in order to measure VF height from lateral view. Rule is in centimeters.

One of the important acoustic variables for voice classification is fundamental frequency (F0), and vocal fold length is the primary variable for adjustment of F0.[9] Therefore, a study of phonatory-related structures should include a comparison of vocal fold lengths in different animals. Two additional anatomic properties, however, interact to determine changes in vocal fold length. Vocal fold height, defined as the distance from the axis of rotation of the cricothyroid joint to the longitudinal axis of the vocal fold, is an index of the mechanical advantage of the thyroid cartilage for the purpose of phonation.[10] Thyroid cartilage rotation is controlled by the combined actions of the cricothyroid and thyroarytenoid muscles. The range of thyroid cartilage rotation, combined with the mechanical advantage of vocal fold height, determines the magnitude of vocal fold elongation for an individual larynx.

Vocal fold oscillation is commonly described as a mass-spring oscillating system. The natural frequency of a mass-spring oscillator is proportional to the square root of the stiffness-to-mass ratio.[11] Vocal fold stiffness can be represented by applying a constant lateral force that causes a translational displacement of the vocal fold.[12] In normal vocal fold vibration, such a displacement determines the size of the glottal aperture and builds up a restoring force against the intraglottal driving force.[6] Vocal fold stiffness is controlled by elongation of the vocal fold; therefore,

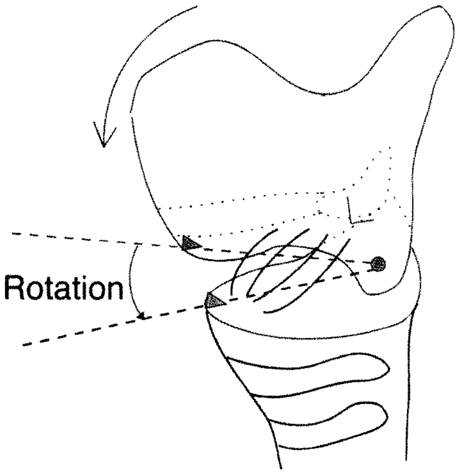

Fig 3. Method of measuring angular range of rotational motion of cricothyroid joint.

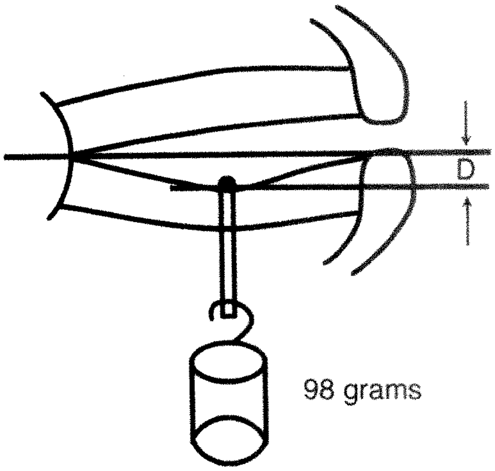

Fig 4. Measurement of force of displacement (D) by 98-g weight applied perpendicularly to long axis of vocal fold.

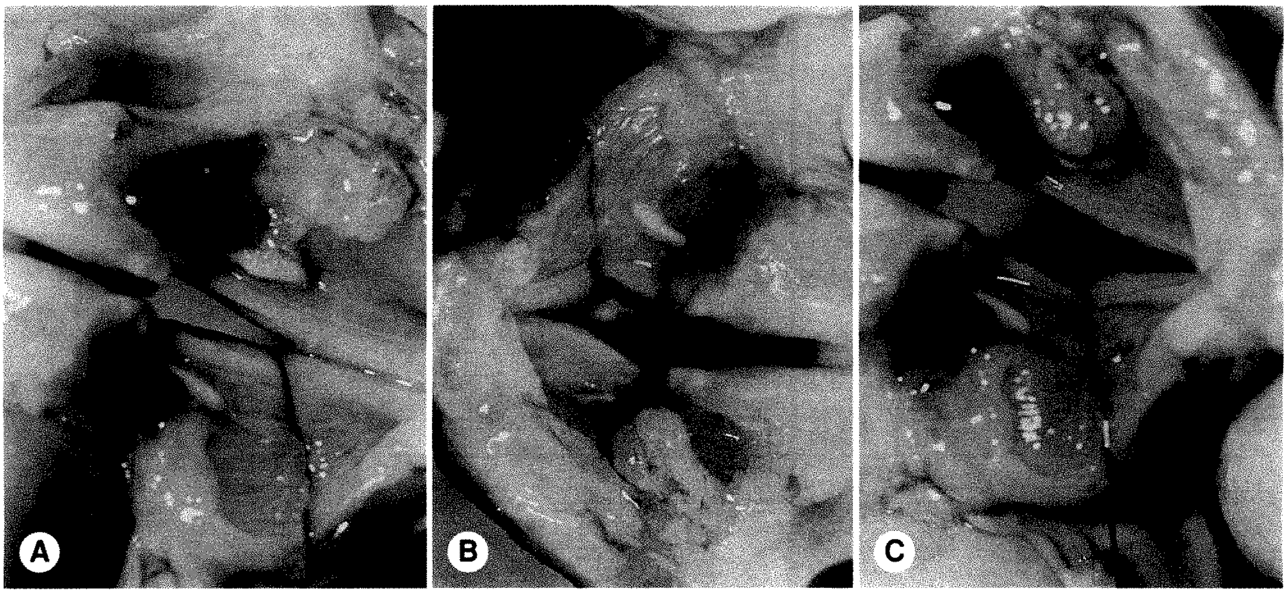

Fig 5. Method of measuring vocal fold stiffness by assessing displacement of vocal fold due to fixed force perpendicular to long axis of vocal fold, at 3 positions: **A**) elongated, **B**) neutral, and **C**) shortened.

the stiffness at different elongations can also be observed.

METHODS

Excised larynges from 2 humans, 3 pigs, 3 dogs, and 3 white-tailed deer were studied. For all excised larynges, remaining strap muscles were trimmed from the thyroid cartilage. The cuneiform cartilage and epiglottis also were removed to better expose the laryngeal cavity.

For each larynx, the resting length of the membranous vocal fold was measured from the anterior commissure to the vocal process of the arytenoid cartilage (Fig 1). The distance from the cricothyroid joint to the longitudinal axis of the vocal fold was also measured as the height of the vocal fold (Fig 2A). A long needle was inserted parallel to the vocal fold body in order to measure the vocal fold height from the lateral view (Fig 2B). The angular range of motion of the cricothyroid joint was measured by applying a constant force (about 1,500 mg) across the joint, in directions toward and against the direction of cricothyroid pull (Fig 3). When no force was applied, the neutral position was determined. Corresponding to the directions of the applied force, the vocal fold had 3 positions: elongated, neutral, and shortened. Vocal fold stiffness was measured by the method described by Isshiki.[12] A constant force, produced by a 98-g weight, was applied perpendicularly to the long axis of the vocal fold (Fig 4). This was done at 3 positions of vocal fold elongation (Fig 5). The original vocal fold length was divided by its lateral displacement to give the index of vocal fold stiffness. This index was then plotted at different levels of vocal fold elongation. Prephonatory glottal configurations were also observed from coronal sections. The variance among the measurements within species was less than 15% in our limited cases; therefore, average values are reported.

RESULTS AND DISCUSSION

Gross anatomic comparisons of the laryngeal frames are shown in Fig 6. The pig larynx (Fig 6A) demonstrates a very large thyroid cartilage and cricothyroid muscle. Figure 6B shows a lateral view of the human male larynx. The dog larynx (Fig 6C) has a smaller thyroid cartilage but a larger cricothyroid muscle than that of the pig. In the deer larynx (Fig 6D), the cricothyroid muscle appears to be smaller and more oblique in orientation. Cricothyroid joint rotation is minimal in deer. Instead, there is some bending of the thin inferior cornu during cricothyroid rotation.

Figure 7A summarizes membranous vocal fold length data for the 4 species. The membranous vocal fold lengths of all larynges were fairly similar, with those of dogs being closest to those of humans. Pig and deer vocal folds were slightly longer.

The heights of the vocal fold above the cricothyroid rotation point were similar in humans, pigs, and dogs, but significantly smaller in deer (Fig 7B). The angles of rotation of the cricothyroid joint, measured from 3 positions, are shown in Fig 8. The positive direction is the same as the force of contraction of the cricothyroid muscle. The ranges of the angles of rotation are summarized in Fig 9. The human, pig, and dog larynges have very similar ranges of mo-

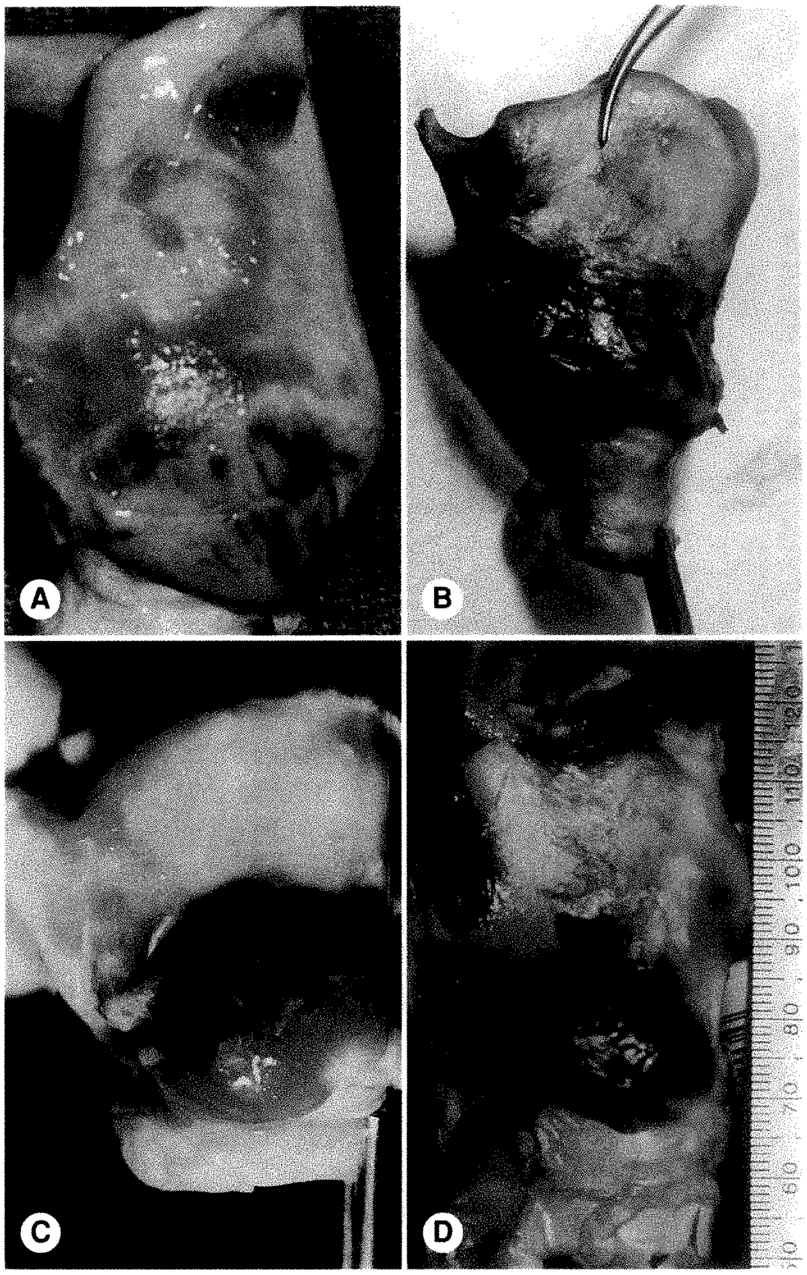

Fig 6. Lateral views of larynges of **A**) pig, **B**) human, **C**) dog, and **D**) deer. Rule is in centimeters.

tion, while that of the deer is more restricted. The increased height of the vocal fold, combined with the greater range of motion at the cricothyroid joint, gives the human, pig, and dog larynges a greater mechanical advantage for vocal fold lengthening.

Figure 10 shows the index of vocal fold stiffness plotted against vocal fold elongation. Increasing displacement is plotted on the vertical axis of the graph, and variations of vocal fold elongation, corresponding to thyroid cartilage rotation, are represented on the horizontal axis. Lateral displacement increases as vocal fold length decreases for all larynges. Less displacement indicates greater stiffness of the vocal fold. The deer and human vocal folds have the greatest lateral displacement at all thyroid cartilage rotational positions. The dog folds have intermediate lateral displacement. The pig folds show relatively small lateral displacement at all rotational positions.

In a previous study,[6] we phonated excised canine larynges at various vocal fold lengths in order to determine the suitable range of elongation for dog phonation (−15% to +30%). We applied this elongation range to the dog plot of vocal fold tension versus vocal fold elongation. The horizontal bars to the right on Fig 10 indicate the range of displacement at tensions within which we would expect to be able to produce phonation in excised dog larynx models. The pig falls within this range for the greatest variation of vocal fold length. Therefore, the pig larynx appears to be a reasonable model for studies of laryn-

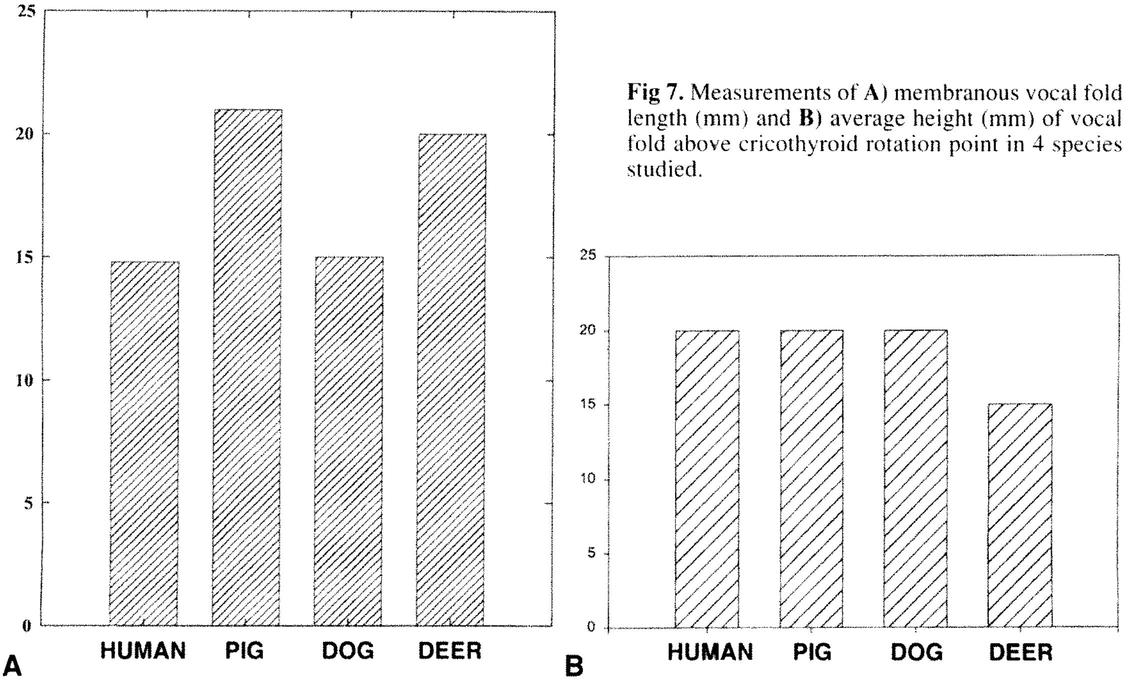

Fig 7. Measurements of **A**) membranous vocal fold length (mm) and **B**) average height (mm) of vocal fold above cricothyroid rotation point in 4 species studied.

geal phonation. The deer vocal folds have relatively large lateral displacement at all degrees of thyroid cartilage rotation. This finding indicates that the deer vocal fold cannot produce a tension for phonation at the frequency of the human voice as the dog larynx does.

The ranges of vocal fold tension probably reflect the sounds that these animals emit naturally. We measured the frequencies of digitized voice recordings that were performed at the San Diego Zoo.[13] The pig emits grunts and squeals that occur over a wide frequency range: 100 to 450 Hz, as measured from the recordings. The dog has a range from a bark to a high-pitched whine. The typical bark we recorded had a frequency of 150 Hz, which is approximately the frequency of the excised dog larynx produced in the laboratory.[6] However, the deer utters only low-pitched, guttural grunts. We obtained a measurement of 61 Hz from the digitized recordings of deer utterances.

CONCLUSION

This study compares 4 species in terms of laryngeal anatomy and function. The vocal fold lengths are similar for all larynges studied. However, the interactions of additional phonation-related structures differ for certain larynges. The cricothyroid muscle is similar in size for human, dog, and pig larynges. The human, dog, and pig larynges also show greater rotational mobility at the cricothyroid joint than does the deer larynx, and this greater rotational mobility

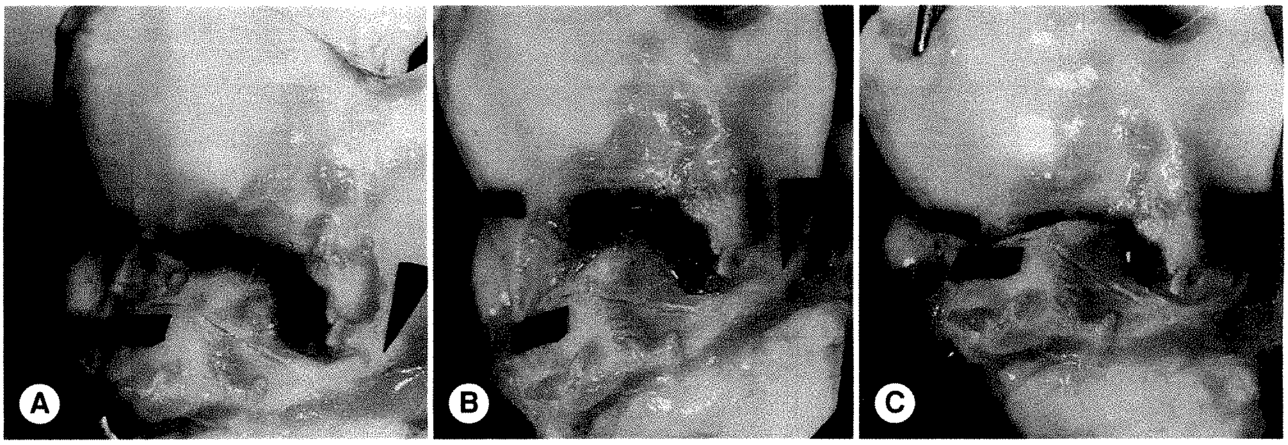

Fig 8. Angle of rotation of cricothyroid joint, measured from 3 rotational positions. **A**) Cricothyroid joint in neutral position. **B**) Joint negatively rotated, parallel to contraction of cricoarytenoid muscle. **C**) Joint positively rotated, parallel to contraction of cricothyroid muscle. Two markers are on thyroid cartilage and cricoid cartilage; they are brought closer together by rotation.

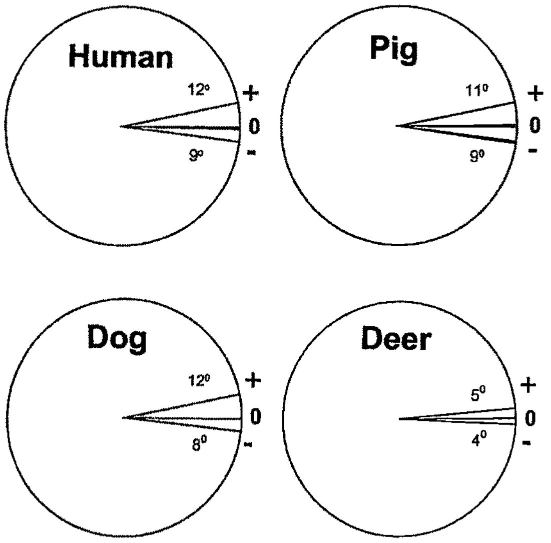

Fig 9. Ranges of angles of rotation summarized in 4 species.

provides for more vocal fold tension in pitch control. The structure of the cartilaginous framework of the larynx also allows for greater mechanical advantage in the human, pig, and dog, but less in the deer. The thickness and structure of the vocal fold cover, and the vocal fold stiffness are most similar in the human and pig larynges. In addition, acoustic analysis of digitized recordings of the natural phonation of the 4 species studied indicates that the F0 and range of phonation in pigs are closest to those of humans.

Although investigators have used the dog larynx extensively as an animal model for the study of vocal physiology, these data suggest, from a structural perspective, that the pig larynx is a superior model.

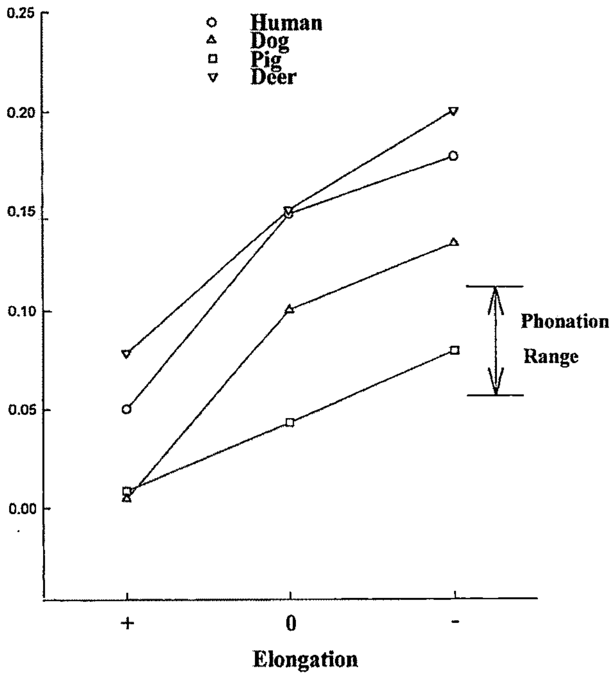

Fig 10. Index of vocal fold stiffness (displacement in centimeters) plotted against vocal fold elongation.

Our data are in agreement with the findings of Kurita et al,[7] which suggest that the pig may provide a reasonable animal model for studies of human phonation through the use of excised larynges. At this stage of our research, we have considered only the suitability of particular excised larynges to serve as phonation models. The nerve supply, the function of laryngeal muscles, and the surgical accessibility have not been considered. Our conclusions do not extend to in vivo models.

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SUPERSELECTIVE EMBOLIZATION AS PALLIATIVE TREATMENT OF RECURRENT HEMORRHAGE IN ADVANCED CARCINOMA OF THE HEAD AND NECK

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We report a case of recurrent major hemorrhage in a patient with advanced head and neck squamous cell carcinoma. Before and between the bleeding episodes, the functional level of the patient was remarkably high. Therefore, an attempt at bleeding control with superselective embolization with Ethibloc was made. Because of its specific characteristics, this substance is almost ideal for the purpose of palliative embolization. The material used and the technique of application are described in detail. After the procedure, no hemorrhage occurred for more than 4 months. We recommend superselective embolization, preferably with Ethibloc, for minimally invasive control of recurrent bleeding as palliative treatment in selected patients with advanced head and neck carcinoma, since significant benefit in terms of the quality of life may result.

KEY WORDS — head and neck carcinoma, hemorrhage, superselective embolization.

INTRODUCTION

Advanced local disease in recurrent inoperable head and neck carcinoma often follows an almost chronic course, allowing these patients to maintain a relatively high functional level. Typically, sudden massive hemorrhage may occur, representing a devastating, often fatal complication. However, there are a considerable number of patients whose bleeding stems from a large, diffuse, ulcerated bed of tumor. In these cases, intermittent episodes of minor hemorrhage may occur at unpredictable intervals, with a disastrous impact on the quality of life of the patient and his or her family. Ligation of the common or internal carotid artery, an established concept of management, is associated with a relatively high mortality rate and occasional hemiplegia.[1] Moreover, because of back-bleeding from the collateral circulation, the hemostatic effect is not lasting in many cases. Superselective angiographic embolization of a bleeding vessel has been shown to be efficient as interventional palliative therapy in many fields of medicine.[2] We present a case of successful superselective embolization using Ethibloc for a repeatedly bleeding tongue base tumor in a patient in otherwise satisfactory general condition.

CASE REPORT

A 56-year-old man had received a diagnosis of T1 glottic carcinoma in 1988. At that time, vertical reconstructive hemilaryngectomy with selective ipsilateral neck dissection had been performed. Postoperative radiotherapy in a curative dosage of 60 Gy had been administered. Follow-up examinations had been continued for 7 years at regular intervals without suspect findings. In March 1999, the patient presented with dysphagia. Subsequent microlaryngoscopy and hypopharyngoscopy revealed a stage IV squamous cell carcinoma of the left pyriform sinus involving the complete circumference of the esophageal orifice caudally and a major portion of the tongue base cranially. After careful discussion of treatment options, the patient decided to undergo surgery. The surgical concept consisted of total laryngopharyngectomy with reconstruction of the pharyngeal passage by a free microvascular anastomosed jejunal graft. Unfortunately, cranial resection margins were not histologically clear, and further cranial resection was not technically feasible. Nevertheless, the postoperative course was uneventful, and the patient returned to an almost normal diet a few weeks later. As anticipated, there were residual tumor masses of increasing size at the base of the tongue, causing progressive dysphagia and requiring laser debulking twice over the following months. Otherwise, the patient's general condition was good and his functional level was remarkably high. In October 1999, massive hemorrhage occurred for the first time. The patient was admitted without active bleeding and with a hemoglobin level of 86 g/L. However, over the next 7 days, 3 more episodes of acute hemorrhage occurred, each stopping spontaneously after several minutes. Each bleeding episode evoked panic in the patient, requiring

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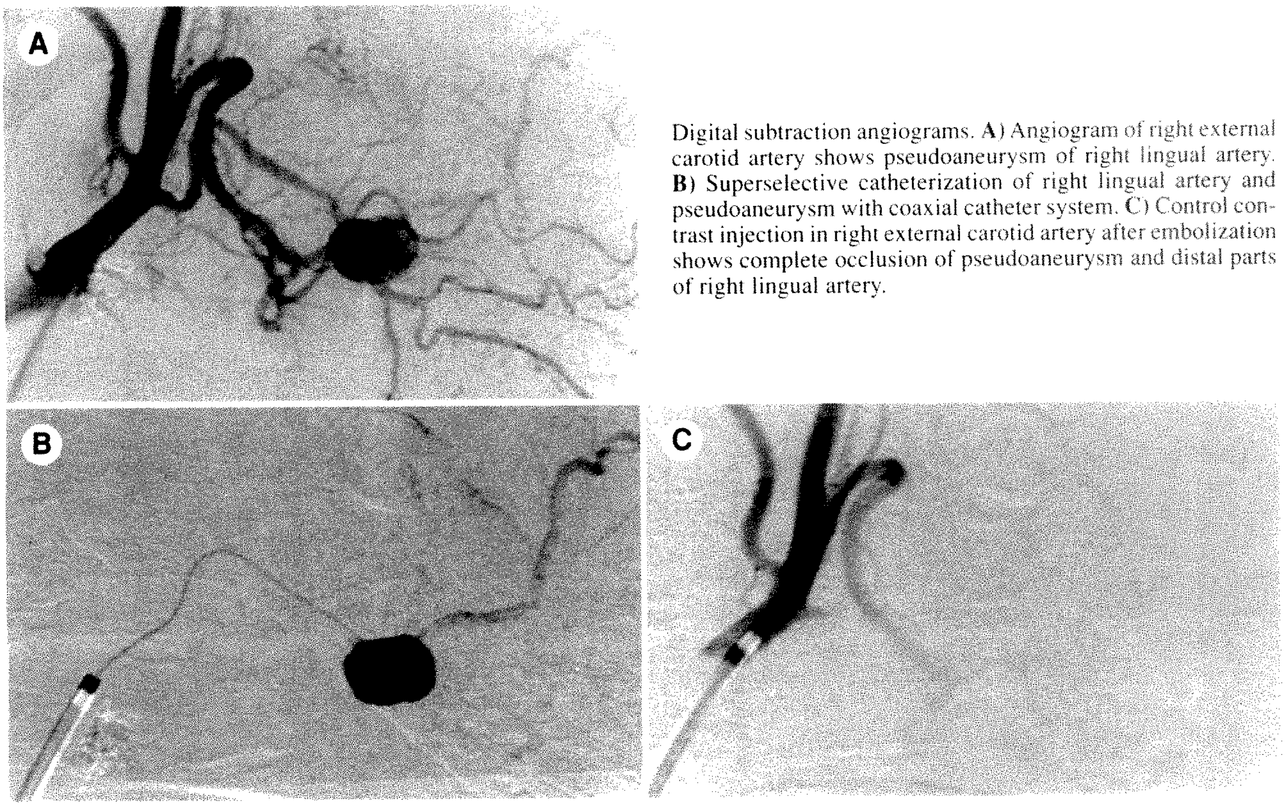

Digital subtraction angiograms. **A**) Angiogram of right external carotid artery shows pseudoaneurysm of right lingual artery. **B**) Superselective catheterization of right lingual artery and pseudoaneurysm with coaxial catheter system. **C**) Control contrast injection in right external carotid artery after embolization shows complete occlusion of pseudoaneurysm and distal parts of right lingual artery.

potent sedation. Still in satisfactory condition, the patient wished the bleeding controlled by any procedure possible. Ligation of the carotid artery was considered to be of even greater risk than usual in this case, because of heavy cervical scar formation following the several surgical procedures and postirradiation tissue fibrosis. Additionally, we were reluctant to put at risk the microvascular anastomosis of the jejunal graft by such a procedure. Therefore, we suggested angiographic superselective embolization for bleeding control, to which the patient agreed after careful discussion.

Digital subtraction angiography was performed by a right-sided unifemoral approach. Selective studies of both external carotid arteries were performed with a 5F Sidewinder-2 catheter (Cordis Europe, Roden, the Netherlands). Angiograms of the external carotid arteries showed a pseudoaneurysm of the right lingual artery (see Figure, A). Superselective catheterization of the right lingual artery and the pseudoaneurysm was then attempted by use of a coaxial catheter system (Meditech, Boston Scientific Corporation; see Figure, B). Subsequently, a mixture of Ethibloc (Johnson & Johnson, Brussels, Belgium) and Lipiodol (Byk Gulden, Konstanz, Germany) was freely introduced with fluoroscopic guidance. Control contrast injections in the right external carotid artery made after embolization showed complete occlusion of the pseudoaneurysm and the distal parts of the right lingual artery (see Figure, C). Nine months later, no new episode of hemorrhage had occurred since embolization.

DISCUSSION

Transcatheter techniques for devascularization are useful in a wide variety of diseases. The role of transcatheter embolization has expanded greatly in recent years with the availability of coaxial microcatheter systems that allow delivery of the embolic agent to the site of treatment with extreme precision.

Embolization is used primarily as a preoperative measure to minimize intraoperative blood loss, and as a primary therapy in arteriovenous malformations[3] and hemangiomas or similar lesions.[4] It is a palliative therapy of increasing importance for metastatic disease in the liver or metastases from hormonally active tumors such as carcinoid.[2]

Bleeding from unresectable or advanced local recurrent carcinoma of the head and neck represents a difficult management problem. In the literature, the focus has been on emergency ligation of the carotid artery to manage massive, life-threatening hemorrhage.[5] Disadvantages of this technique are its relatively high mortality rate, occasional hemiplegia, and rebleeding due to backflow from collateral circulation. Hence, the risk-benefit ratio is often not easy to determine in a palliative setting. From our point of view, any procedure with the potential of causing additional harm or further reducing the quality of life can be of only limited use for an individual suffering from incurable disease in its terminal phase.

In recent years, some reports on minimally invasive endovascular management of acute hemorrhage from advanced head and neck carcinoma were published.[6-8] Innovations with guiding catheters and microcatheters have improved access to vascular lesions of the head and neck. In general, there are 2 basic types of microcatheters used today: flow-directed and over-the-wire. Besides the type of the microcatheter, the size of the catheter also plays a critical role. Although smaller microcatheters are able to advance into smaller vessels, their luminal diameter limits the size of the embolic agents used.[9,10] A variety of materials are used for embolization: liquid embolic agents such as Ethibloc and *N*-butyl cyanoacrylate, particulate agents such as polyvinyl alcohol particles, and microcoils. In the described case, we used Ethibloc for the embolization of a pseudoaneurysm. Ethibloc is an amino acid gel that acts by solidifying shortly after exposure to an ionic environment. It forms a permanent cast of the vascular bed and causes fibrosis by local irritation. Ethibloc is a viscous substance, so microcatheter administration is difficult, and its primary use is in percutaneous procedures involving capillary and venous angiomas of the head and neck.[9] Consistent with that of other groups,[9] our experience suggests that the viscosity of Ethibloc assists in preventing this agent from flowing out of the lesion, and that its radiopacity allows for visualization during injection. Therefore, the whole lumen of a pseudoaneurysm and the feeding artery can be embolized completely under fluoroscopic guidance. Embolic complications are comparatively rare because of the viscosity of Ethibloc. This profile makes Ethibloc an almost ideal agent to serve the purpose of palliative embolization. By contrast, particulate agents have the ability to penetrate into small vessels down to a diameter of 40 to 60 μm. Particles with sizes in the 40- to 140-μm range, however, should be used with caution, because these have a greater propensity to cause severe ischemia of normal tissue.[10] Moreover, particulate agents act as temporary occlusive agents.[9] Particulate agents therefore, are, not useful for the embolization of a pseudoaneurysm. Balloons and microcoils allow for permanent occlusion of larger vessels. The main disadvantage of these techniques are the relatively high costs compared with Ethibloc. In general, the risks of embolization include strokes, blindness, seizure, hemorrhage, and thrombosis. Fatal complications have been reported, but are generally thought to be uncommon.[2] Arterial embolization may be advantageous over ligation in terms of bleeding control, since distal migration of the embolizing material also occludes collateral feeding vessels. To the best of our knowledge, this is the first report on the use of Ethibloc for palliative bleeding control in advanced head and neck carcinoma.

There is no doubt that advanced head and neck cancer patients frequently have a very low quality of life. Exsanguination may be one of the less horrifying forms of death in such a situation. There is also no doubt that intra-arterial embolization does not change the ultimate prognosis in this patient population. On the other hand, in carefully selected patients who are maintaining a relatively high functional level that is compromised by recurrent hemorrhage, we strongly believe that endovascular embolization can be most helpful in enabling these patients to spend their remaining time in dignity and relatively free of fear.

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MATRIX METALLOPROTEINASE–1 AND TISSUE INHIBITOR OF METALLOPROTEINASE–1 GENE EXPRESSIONS AND THEIR DIFFERENTIAL REGULATION BY PROINFLAMMATORY CYTOKINES AND PROSTAGLANDIN IN NASAL POLYP FIBROBLASTS

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Chronic inflammation of the paranasal sinus leads to nasal polyp (NP) formation. In this study, we investigated the effect of stimulation of the proinflammatory cytokines interleukin-1α (IL-1α) and tumor necrosis factor–α (TNF-α) and prostaglandin (PG) E2 on the production of messenger RNA (mRNA) of matrix metalloproteinase–1 (MMP-1) and tissue inhibitor of metalloproteinase–1 (TIMP-1) in nasal polyp fibroblasts (NPFs) and nasal mucosa fibroblasts (NFs). The mRNAs of IL-1α, TNF-α, MMP-1, and TIMP-1 in 40 surgical specimens of NPs were studied by in situ hybridization to corroborate the in vitro findings. The results indicated a significant amount of constitutive MMP-1 mRNA in NPFs and cytokine-induced MMP-1 steady-state mRNAs in NFs. The effect of stimulation of cytokines on TIMP-1 mRNA synthesis was unremarkable in NPFs and NFs. Exogenous PGE2 enhanced cytokine-stimulated MMP-1 mRNA synthesis in NPFs. In situ hybridization revealed that cells expressing MMP-1 and TIMP-1 mRNAs (primarily plasma cells, fibroblasts, and endothelial cells) gathered around areas with loose stroma, suggestive of rapid extracellular matrix degradation. These data suggest that the pathogenesis of nasal polyposis could be related to production of MMP-1 and consequent promotion of matrix collagenolysis.

KEY WORDS — interleukin-1α, matrix metalloproteinase–1, nasal polyp, prostaglandin, tissue inhibitor of metalloproteinase–1, tumor necrosis factor–α.

INTRODUCTION

Nasal polyposis, a chronic inflammatory disease of the nasal mucosa, is the most common mass lesion encountered in the practice of otolaryngology. However, the mechanism controlling the formation and growth of nasal polyps (NPs) remains poorly understood. Previous studies have suggested that the formation and growth of NPs require an active remodeling of the lamina propria, which includes fibroblasts and the extracellular matrix (ECM).[1,2] Fibroblasts, the main cellular source of ECM proteins, may therefore play an important role in the development and growth of NPs.

The matrix metalloproteinases (MMPs), a family of zinc-dependent endopeptidases with proteolytic activities toward several components of the ECM, are involved in many physiological and pathological aspects of remodeling of connective tissue. Of the MMP family, interstitial collagenase (matrix metalloproteinase–1 [MMP-1], EC 3.4.24.7) is the key enzyme responsible for degrading collagen, as the initial breakdown of the fibrillar collagen network is mediated primarily by MMP-1.[3,4]

The activity of MMP-1 is under strict regulation by a group of tissue inhibitors of metalloproteinases (TIMPs), of which TIMP-1 to TIMP-4 have been identified.[5] TIMP-1, a glycoprotein with a molecular mass of 28 kd, can form a stoichiometric 1:1 complex with and inactivate MMP-1.[3,5] Since the same cells that produce MMP-1 are also capable of synthesizing TIMP-1, the overall molar ratio between MMP-1 and TIMP-1 plays a significant role in connective tissue remodeling.[6]

Proinflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor–α (TNF-α) may initiate the inflammatory process. Studies have indicated that both infiltrating and resident cells may augment the inflammatory cascade by releasing MMP-1 and TIMP-1.[3,4] Studies have proposed roles for IL-1,[7] TNF-α,[8] and prostaglandins (PGs) in the pathogenesis of NPs; however, the subsequent reactions leading to NP development following the induction of IL-1 or TNF-α remain unclear. It is well established that IL-1 and TNF-α can mediate inflammatory tissue destruction by stimulating MMP-1 and TIMP-1 production in several cell types.[9,10] In addi-

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tion, PGs, the metabolites of cell membrane arachidonic acid, further complicate the inflammatory reaction by modulating some biological effects of IL-1 and TNF-α.[11,12] The mechanism(s) underlying the synthesis of MMP-1 and TIMP-1 in nasal polyp fibroblasts (NPFs) is still unknown. Furthermore, the regulatory role of PGs on cytokine-stimulated MMP-1 and TIMP-1 gene expression has never been elucidated. This may be of clinical relevance, since NPFs are most likely to be exposed to multiple regulatory factors, rather than a single stimulant, during inflammation. To provide further insight into the complex cascade associated with inflammation-mediated tissue remodeling, we investigated in this study the stimulatory effects of IL-1 or TNF-α, as well as the regulatory roles of PGs, on MMP-1 and TIMP-1 gene expression in NPFs. In addition, the in vivo distribution of IL-1α, TNF-α, MMP-1, and TIMP-1 gene expression in NPs was elucidated by in situ hybridization for correlation with the in vitro findings.

MATERIALS AND METHODS

Tissue Samples. Nasal mucosal tissues were obtained from the inferior turbinates of patients who were undergoing turbinectomy to relieve nasal obstruction. Before surgery, the tissue appeared to be free of inflammation and possessed a normal appearance. Nasal polyps were resected by functional endoscopic sinus surgery for treatment of chronic sinusitis with polyposis. The patients had no history of nasal allergy, asthma, or aspirin sensitivity and had not regularly taken topical or oral medication within 3 weeks. Informed consent was obtained before the surgical procedure.

Cell Culture. After removal of the epithelial layer, the tissues were immersed in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum, 200 U/mL penicillin, 200 μg/mL streptomycin, and 1 mg/mL amphotericin B. After overnight incubation, the samples were placed in a 10-cm Petri dish, minced into 1-mm^3 fragments, and covered with a sterilized glass coverslip. The tissue explants were incubated with DMEM supplemented with 10% fetal calf serum, 100 U/mL penicillin, 100 μg/mL streptomycin, and 0.5 mg/mL amphotericin B under 5% carbon dioxide at 37°C until confluence. They were detached with 0.05% trypsin and subcultured.

Cell Stimulation. Cells between passages 3 and 6 were plated at a density of 5×10^5/mL on a 10-cm Petri dish. They were incubated in serum-free medium for 6 hours and stimulated with the following reagents: IL-1α (10 ng/mL), TNF-α (10 ng/mL), PGE2 (1 μg/mL), IL-1α and PGE2, TNF-α and PGE2. After an incubation time of 24 hours, total RNAs were harvested for analysis. To further elucidate the role of PGs on MMP-1 and TIMP-1 gene expression, we preincubated cells with 10^{-5} mol/L indomethacin, a concentration that has been proven to block endogenous PG synthesis,[9] for 2 hours and subsequently stimulated them with IL-1α and TNF-α. The total RNAs were collected for analysis after 24 hours of treatment.

Probe Preparation. For the Northern blot technique, we used DNA plasmids of human MMP-1 and TIMP-1 that were generous gifts from Dr Barry L. Marmer (Washington University, St Louis, Missouri). Clones were radiolabeled with [^{32}P] deoxycytidine 5'-triphosphate (dCTP) by the random prime labeling method (Amersham, Little Chalfont, England). For in situ hybridization, the probes were labeled with digoxigenin-11-deoxyuridine 5'-triphosphate (DIG-11-dUTP) through random priming (Boehringer Mannheim, Indianapolis, Indiana).

Northern Blot Analysis. The total RNAs within the fibroblasts were isolated by the acid guanidinium thiocyanate–phenol–chloroform method (Zol-B, Biotecx Laboratories, Houston, Texas) and subjected to 0.8% agarose gel electrophoresis. After being electrophoretically transferred to a nylon membrane, the RNAs were immobilized by ultraviolet cross-linking at 1,800 kJ. The membrane was hybridized with radiolabeled complementary DNA (cDNA) probes for MMP-1, TIMP-1, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, as internal standard), washed under a stringent protocol, and autoradiographed with an intensifying screen (XAR-5 film, Eastman Kodak, Rochester, New York) at −80°C. The intensity of each band, after normalization with GAPDH messenger RNA (mRNA), was quantitated by a scanning video densitometer and software (Biomed Instrument, Fullerton, California). Each densitometric value, expressed as mean ± SD, was obtained from 3 independent experiments.

In Situ Hybridization. Forty surgical specimens of NPs and 15 specimens of nasal mucosa were collected. They were fixed in 4% paraformaldehyde and embedded in paraffin. Serial sections of 5 μm were cut and mounted on Superfrost/Plus slides (Fisher Scientific, Pittsburgh, Pennsylvania). The sections were deparaffinized, rehydrated, and treated with 0.1% pepsin–0.2N hydrochloric acid. They were postfixed with 0.4% paraformaldehyde and acetylated with acetic anhydride. The sections were then prehybridized with hybridization buffer (4X sodium chloride and sodium citrate buffer [SSC], 50% deionized formamide, 1X Denhardt's solution, 5% dextran sulfate, 0.5 mg/mL salmon sperm DNA, and 0.25 mg/mL yeast total RNA). Hybridizations were carried out overnight with hybridization buffer containing

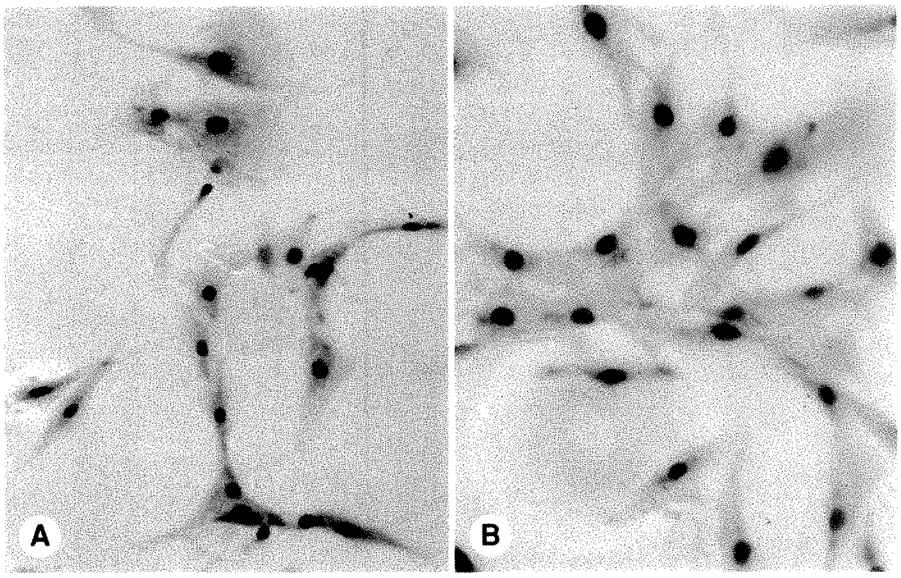

Fig 1. Cells cultured from **A)** nasal polyps and **B)** inferior turbinate mucosa exhibited spindle shape similar to that of fibroblasts.

0.5 to 1.0 µg/mL digoxigenin-labeled probes at 42°C. After a series of washings in SSC at decreasing concentrations, the sections were incubated with buffer 1 (0.1 mol/L Tris–hydrochloride [Tris-HCl] pH 7.5, 0.1 mol/L maleic acid, 0.15 mol/L sodium chloride, and 0.3% Triton X-100). Slides were incubated with alkaline phosphatase–conjugated sheep anti-digoxigenin antibody overnight at 4°C, washed with buffer 1, and rinsed in buffer 2 (0.1 mol/L Tris-HCl pH 9.5, 0.1 mol/L sodium chloride, and 0.05 mol/L magnesium chloride). Hybridization signals were visualized by incubating the slides in a chromogen solution (buffer 2 containing 0.45% 4-nitroblue tetrazolium and 0.35% 5-bromo-4-chloro-3-indolylphosphate). The sections were then counterstained with methyl green, mounted with Permount, and examined by light microscopy. Sections digested with RNaseA before hybridization served as negative controls.

RESULTS

Cells cultured from the NPs and inferior turbinates exhibited a spindle shape similar to that of fibroblasts and were subcultured in 10% fetal calf serum–supplemented DMEM (Fig 1).

Different MMP-1 Gene Expression Patterns in NPFs and NFs Following IL-1α and TNF-α Stimulation. After exposure to various stimuli for 24 hours, total RNAs in NPFs and nasal mucosa fibroblasts (NFs) were extracted and subjected to Northern blot hybridization. Densitometric analysis of the blots was performed after normalization by GAPDH messenger RNA (mRNA), as shown in Figs 2-4. Exposure of NFs to IL-1α and TNF-α resulted in elevated (approximately 13- and 6.7-fold) levels of MMP-1 steady-state mRNAs as compared with the control groups (Figs 2 and 4). The NPFs acted differently from NFs in that they produced a prominent amount of constitutive MMP-1 mRNA and the gene level was not upregulated by IL-1α or TNF-α.

TIMP-1 mRNA Constantly Expressed in NPFs and NFs. Cytokines (IL-1α or TNF-α) and PGE2, either alone or in combination, did not stimulate significant production of TIMP-1 mRNA as compared with the control groups.

Diverse Effects of PGE2 Administration on Cytokine-Stimulated MMP-1 Gene Expression in NFs and NPFs. Densitometric analysis revealed that IL-1α and TNF-α upregulated NF MMP-1 mRNA approximately 13-fold and 6.7-fold, respectively. PGE2 reduced the IL-1α–stimulated, but not the TNF-α–stimulated, MMP-1 mRNA synthesis in NFs. On the other hand, concomitant addition of cytokine (IL-1α or TNF-α) and PGE2 to NPFs resulted in significant elevation (approximately 2.2-fold) of the MMP-1 mRNA level as compared with stimulation by a cytokine alone.

Modulating Effects of Endogenous PG Blocker, Indomethacin, on MMP-1 and TIMP-1 Gene Expression. Indomethacin failed to modify the MMP-1 and TIMP-1 mRNA levels stimulated by IL-1α or TNF-α in both NPFs and NFs as compared with stimulation by a cytokine alone (Figs 2-4).

Similar Distribution Patterns of MMP-1, TIMP-1, IL-1α, and TNF-α mRNAs. Under microscopic examination, the NPs were lined by a layer of ciliated respiratory epithelium. There was significant variation concerning the consistency of the ECM, even in the same lesion (Fig 5). Pronounced MMP-1 mRNAs presented as dark brownish granules and were detected in the cytoplasm of plasma cells, fibroblasts, and endothelial cells. The lining epithelium, how-

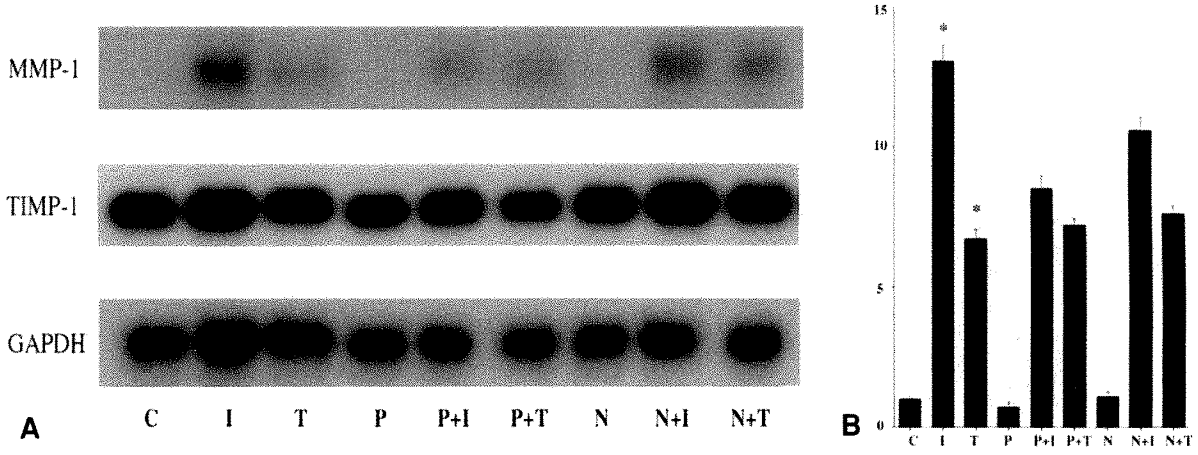

Fig 2. A) Induction of matrix metalloproteinase–1 (MMP-1) and tissue inhibitor of metalloproteinase–1 (TIMP-1) messenger RNA (mRNA) transcripts by interleukin-1α (IL-1α), tumor necrosis factor–α (TNF-α), and prostaglandin E_2 (PGE_2) in nasal mucosa fibroblasts (NFs). Cells were untreated (C) or treated for 24 hours with IL-1α (10 ng/mL; I), TNF-α (10 ng/mL; T), or PGE_2 (1 μg/mL; P). In addition, some cells were preincubated with indomethacin (10^{-5} mol/L) for 2 hours before subsequent stimulation by indomethacin (10^{-5} mol/L; N), IL-1α (N+I), or TNF-α (N+T) for 24 hours. Total RNAs were collected and subjected to analysis of MMP-1 and TIMP-1 by Northern blot hybridization. **B)** Results from Northern blot analysis were quantified by densitometric analysis after glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA standardization and expressed as optical density relative to that of control group (C). Each bar represents mean ± SD of 3 experiments. Asterisks — $p < .05$ compared to control (Student's *t*-test).

ever, revealed no MMP-1 signals. Furthermore, there seemed to be a correlation between the distribution of MMP-1 genes and the ECM consistency. Prominent MMP-1 mRNAs tended to accumulate in areas with loose stroma, whereas only faint signals could be identified in areas composed of relatively "compact" ECM. Also, TIMP-1 mRNAs were located in plasma cells and fibroblasts. However, most cellular components appeared to produce relatively weaker TIMP-1 mRNA in contrast to the significant transcription of the MMP-1 gene. Marked IL-1α and TNF-α mRNAs were also identified in NPs with cellular sources similar to MMP-1 and TIMP-1 (data not shown). On the other hand, expression of MMP-1 and TIMP-1 genes was insignificant in nasal mucosa from the inferior turbinate. Little or no signal was found in the negative control groups.

DISCUSSION

Nasal polyps are non-neoplastic growing masses

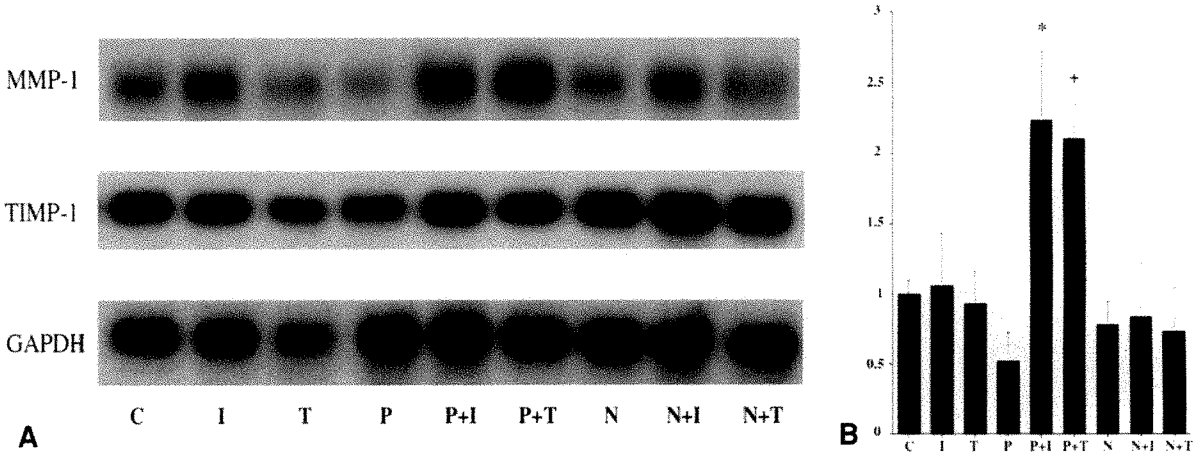

Fig 3. A) Induction of MMP-1 and TIMP-1 mRNA transcripts by IL-1α, TNF-α, and PGE_2 in nasal polyp fibroblasts (NPFs). NPFs were untreated (C) or treated for 24 hours with IL-1α (10 ng/mL; I), TNF-α (10 ng/mL; T), or PGE_2 (1 μg/mL; P). In addition, some cells were preincubated with indomethacin (10^{-5} mol/L) for 2 hours before subsequent stimulation by indomethacin (10^{-5} mol/L; N), IL-1α (N+I), or TNF-α (N+T) for 24 hours. Total RNAs were collected and subjected to analysis of MMP-1 and TIMP-1 by Northern blot hybridization. **B)** Results from Northern blot analysis were quantified by densitometric analysis after GAPDH mRNA standardization and expressed as optical density relative to that of control group (C). Each bar represents mean ± SD of 3 experiments. Asterisk — $p < .05$ compared to IL-1α group (I); cross — $p < .05$ compared to TNF-α group (T) (Student's *t*-test).

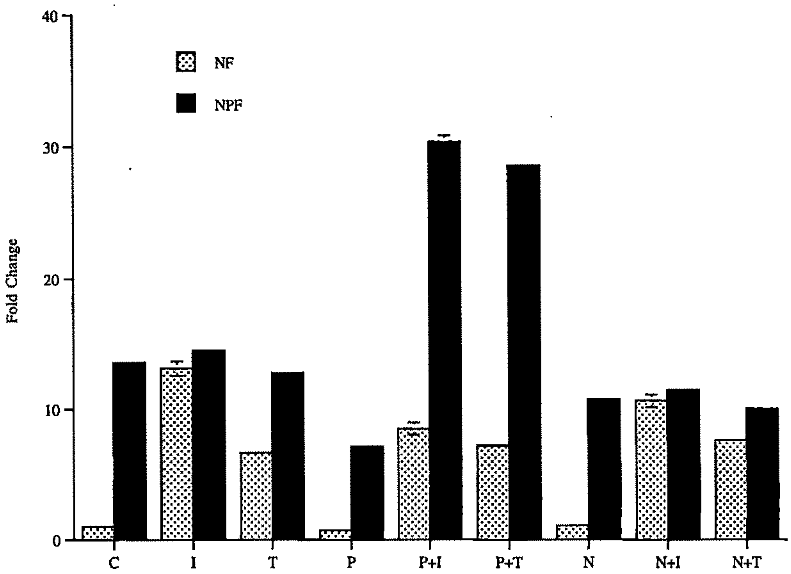

Fig 4. After various stimuli described in Figs 2 and 3, MMP-1 mRNAs synthesized in NFs and NPFs were subjected to densitometric analysis, normalized by GAPDH mRNA, and expressed as optical density relative to that of NF control group.

that usually arise from the middle meatus and ethmoid sinuses, but never occur in the inferior turbinates.[2] Active ECM remodeling in this lesion is necessary to support its rapid proliferation. In this experiment, we showed that there was an obvious amount of constitutive MMP-1 mRNA in NPFs. Interleukin-1α and TNF-α induced a significant elevation of the MMP-1 steady-state mRNA level in NFs, but not in NPFs. The stimulation by a cytokine of TIMP-1 mRNA synthesis was unremarkable in NPFs and NFs. Exogenous PGE2 enhanced the cytokine-stimulated MMP-1 mRNA synthesis in NPFs as compared with the effect of a cytokine or PGE2 alone. Indomethacin, the endogenous PG blocker, failed to modify IL-1, TNF induction of MMP-1, or TIMP-1 gene expression in either kind of cell. In situ hybridization revealed the main cellular sources for MMP-1 and TIMP-1 mRNAs to be plasma cells, fibroblasts, and endothelial cells. These cells tended to accumulate in areas with rather loose stroma. Furthermore, the intensity of TIMP-1 gene expression was obviously weaker than that of MMP-1.

Matrix metalloproteinase–1 plays the role of the key enzyme in ECM remodeling through degrading the native triple helix of collagen and rendering it sensitive to further digestion by other proteinases.[3,4] The high amount of constitutive MMP-1 mRNA detected in NPFs implies active ECM remodeling in NPs. The findings reflect the study done by Larsen and Tos,[2] who hypothesized that rapid ECM degradation may be necessary to support the growth and elongation of mucoserous glands and subsequent NP proliferation. The marked difference in MMP-1 gene intensity between the NPs and nasal mucosa may further elucidate the in vivo significance of MMP-1 in NP progression. This finding, furthermore, may partially explain why most NPs present as diffuse polyposis and have a high recurrence rate.

Indomethacin failed to alter the MMP-1 mRNA level in NPFs stimulated by IL-1α or TNF-α. This finding suggests that the cytokine effect on NPF MMP-1 gene expression is not dependent on endogenous PGs, or else that these PGs are not sufficient to affect the stimulatory activity of cytokines. By modulating adenyl cyclase activity to increase the intracellular level of cyclic adenosine monophosphate (cAMP), PGs, especially the E series, provoke a wide range of biological activities in diverse tissues. Although a cytokine or PGE2 alone fails to induce significant synthesis of MMP-1 in NPFs, concomitant PGE2 and IL-1α or TNF-α demonstrate a pronounced synergistic effect. A similar phenomenon has been reported in cultured human monocytes, in which the cAMP elevating agents (PGE2 or dibutyryl adenosine 3',5'-cyclic monophosphate [Bt2cAMP]) greatly potentiated cytokine-induced MMP-1 production.[13] An explanation for why the combination produced this effect may be that in NPFs, an appropriate primary stimulus is required to activate another transcription pathway that is cAMP-related and also capable of augmenting the PGE2 stimulation of MMP-1 gene expression. To the best of our knowledge, the modulating role of PGE2 on the induction of MMP-1 by cytokines in NPFs has never been demonstrated before. Since NPFs are exposed to the pool of cytokines and PGs during inflammation, our findings elucidate the potential benefits of PG inhibitors such as nonsteroidal anti-inflammatory drugs and anti-cytokine reagent in suppressing the progression of NPs. Furthermore, it is also interesting to note the characteristic differences between NPFs and NFs in their baseline and stimulated MMP-1–producing activities. Since the NFs were obtained from the inferior turbinate, where NPs never occur, more detailed studies

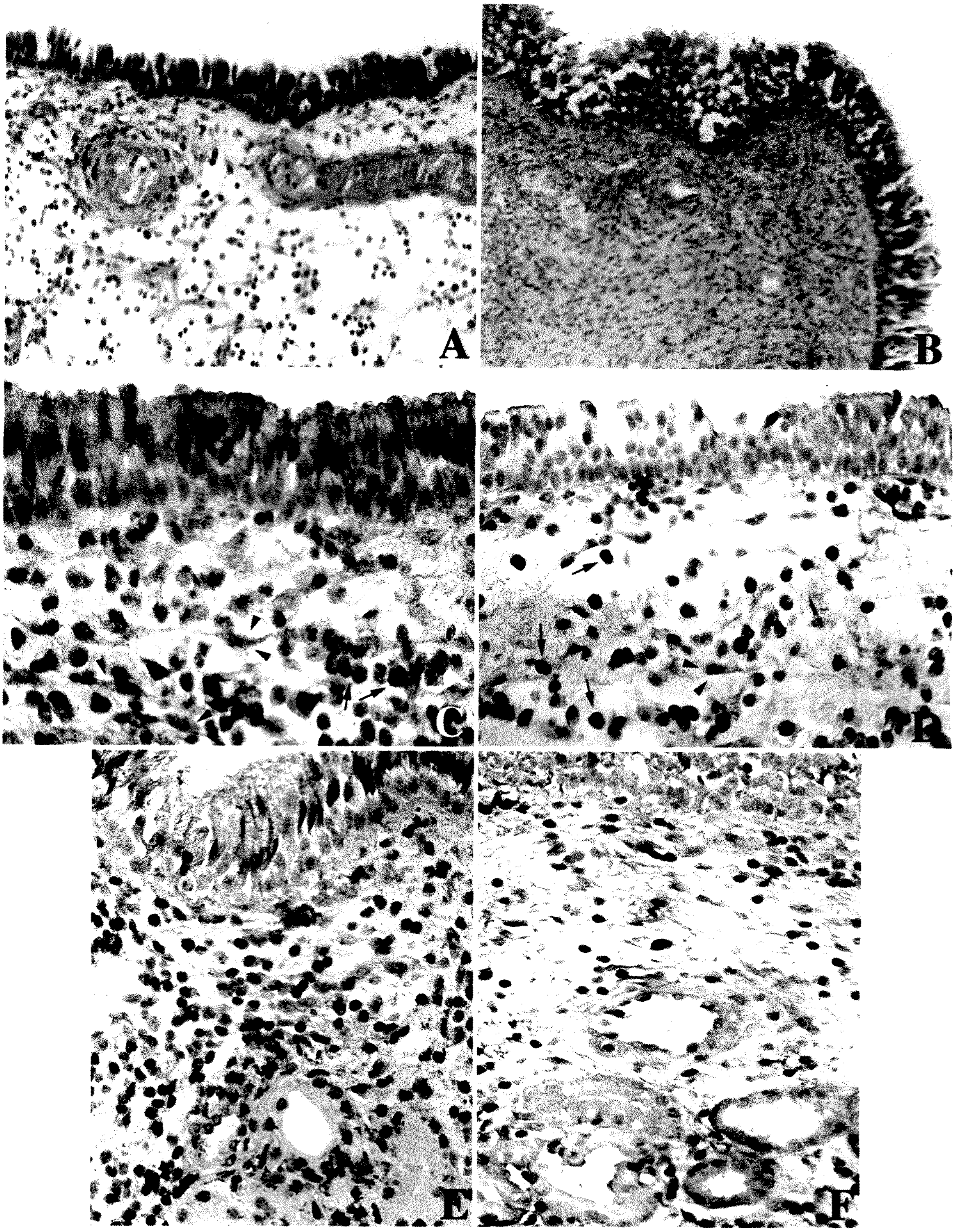

Fig 5. Photomicrographs show that even in same lesion, subepithelial stroma of NP appears rather loose in some areas (**A**) but very dense in other regions (**B**). MMP-1 (**C**) and TIMP-1 (**D**) mRNAs are identified in cytoplasm of plasma cells (arrows), fibroblasts (arrowheads), and endothelial cells, although with different expression intensities. In NFs, expression of MMP-1 (**E**) and TIMP-1 (**F**) genes is insignificant. **A,B**) H & E, original ×150. **C**) In situ hybridization, original ×600. **D-F**) In situ hybridization, original ×300.

on the genetic differences between NPFs and NFs may be helpful in understanding the pathogenesis of NPs.

Being able to antagonize either the active or zymogen form of different MMPs, TIMPs appear to be the major local inhibitor of MMPs. In contrast to MMP-1, neither cytokines nor PGE2 upregulate TIMP-1 gene expression in NFs or NPFs. The differential regulatory effects of cytokine and PGE2 on MMP-1 and TIMP-1 production in NPFs result in an altered balance between these molecules that further triggers active, pathological ECM degradation.

In situ hybridization revealed a similar distribution pattern of MMP-1, TIMP-1, IL-1α, and TNF-α mRNAs. The main cellular sources for these mediators were plasma cells, fibroblasts, and endothelial cells, but not lining epithelium. These MMP-1 mRNA–producing cells tend to accumulate around areas with loose stroma that suggests rapid ECM degradation. In contrast to the faint signals identified in areas composed of relatively "compact" ECM, the observation implies the in vivo collagenolytic role of MMP-1 in NPs. In addition, most cellular components appear to produce relatively fewer TIMP-1 mRNAs, in contrast to the pronounced transcription of MMP-1 genes. These findings may, to certain degree, be consistent with the data shown from in vitro studies demonstrating the differential modulating effects of cytokine and PGE2 on MMP-1 and TIMP-1 gene expression in NPFs.

There are several other factors that contribute to the formation of nasal polyposis other than the abovementioned, including the effect of cytokines on vascular endothelial adhesion molecules, the upregulation of integrin CD49d (very late active protein 4) on eosinophils, the significant influx of eosinophils, the role of bacteria, the role of fungus, and the significant alterations in the cytokine architecture of epithelium in nasal polyposis.[14-23] In this study, the focus was on proinflammatory cytokines and PGs, and the data suggest the involvement of NPFs in the pathogenesis of nasal polyposis through synthesizing MMP-1 to promote matrix collagenolysis. Prostaglandin E2 may further aggravate the disease process through its upregulation of cytokine-induced MMP-1 mRNA production. The unbalanced production of MMP-1 and TIMP-1 in NPFs due to the differential regulation of cytokines and PGE2 also contributes to NP progression. These data may lead us to a better understanding of the pathogenesis of nasal polyposis.

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NONTRAUMATIC ATLANTOAXIAL ROTARY SUBLUXATION IN THE PEDIATRIC OTOLARYNGOLOGY PATIENT

A REPORT OF FOUR CASES

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Nontraumatic atlantoaxial rotary subluxation (NAARS) is a relatively uncommon entity, with inconsistent presentations. It most commonly follows infectious processes or operative procedures. We present our experience with 4 pediatric otolaryngology patients with NAARS who were treated at the University of Iowa Hospitals and Clinics during a 2-year period beginning in 1997. A review of the symptoms, physical findings, and radiographic abnormalities is presented. Treatment options, varying from muscle relaxants to surgical fusion, are discussed. A high index of suspicion in evaluating children with a stiff neck or pain on attempted motion is essential in order to facilitate prompt diagnosis and appropriate intervention.

KEY WORDS — atlantoaxial rotary subluxation, Grisel's syndrome.

INTRODUCTION

Nontraumatic atlantoaxial rotary subluxation (NAARS) associated with head and neck disease processes or otolaryngological procedures is a rare entity. Originally described in 1830 by Bell, NAARS was noted first in a male patient who died as a result of complications of a pharyngeal ulcer.[1] Autopsy demonstrated erosion of the transverse ligament of the axis. Grisel,[2] a French otolaryngologist, later described NAARS as a syndrome that may arise as a complication of an upper respiratory tract infection, a retropharyngeal or cervical abscess, or a surgical procedure such as adenoidectomy. Recognition of the clinical and radiographic signs of this complication is essential if prompt therapy is to be instituted. In this paper, we present 4 cases of NAARS, 1 developing in a young child with a retropharyngeal abscess or phlegmon (Grisel's syndrome), and the other 3 arising in children as complications of operative procedures.

CASE REPORTS

Case 1. A 7-year-old girl with a 5-day history of viral upper respiratory tract illness presented with fever, neck pain, torticollis, odynophagia, and fullness in her neck on the left side. Physical examination confirmed level II adenopathy without fluctuance or inflammatory changes of the overlying skin. Torticollis was present, with the head tilted to the left and the chin rotated to the right. No abnormalities were seen in the upper aerodigestive tract, and the neurologic examination findings were normal. A complete blood cell count with differential revealed leukocytosis with a left shift. Increased fullness of the neck developed despite initiation of intravenous antibiotic therapy, whereupon she was transferred to our institution.

Computed tomography (CT) of the neck suggested abscess formation in the left retropharynx with involvement of the parapharyngeal space (Fig 1). The scan also showed rotary subluxation of C1 on C2. The patient was taken to the operating room for incision and drainage of the abscess and halo placement. A phlegmon was identified in the left retropharyngeal and parapharyngeal spaces, and a Penrose drain was placed, maintaining midline cervical stabilization. At the conclusion of the procedure, a halo ring with 7 pounds of cervical traction was applied. Intravenous antibiotics were continued for 7 days, followed by 1 week of oral antibiotics. After adequate reduction was achieved, the halo ring was attached to a vest, and cervical spine immobilization was maintained for 3

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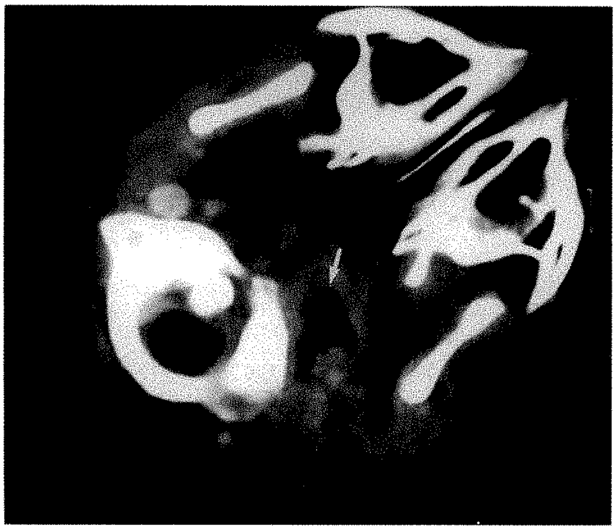

Fig 1. (Case 1) Computed tomogram of 7-year-old with left retropharyngeal abscess. Shows rotary subluxation of C1 on C2 (arrow).

months. A Philadelphia collar was used for 2 weeks after the halo was removed. Follow-up cervical spine films demonstrated normal bony alignment, and the child was released to perform normal activities.

Case 2. A 9-year-old boy presented for evaluation of a left posterior neck mass that had been present for 9 months. The mass measured 8 × 6 cm and was soft and nontender. The child had full range of motion of his neck and did not demonstrate any focal or lateralizing signs. The findings of fine-needle aspiration were consistent with a lipoma, a diagnosis supported by magnetic resonance imaging (MRI). During surgery, the patient was placed in the supine position, and the mass, which was attached to the lateral/transverse processes of C1 and C2, was removed. After the operation, the patient was encouraged to perform a full range of neck exercises. The drain was removed on postoperative day 2, and the patient was discharged on postoperative day 3 with instructions to refrain from contact sports. He returned for suture removal 1 week after surgery and was noted to have decreased neck mobility; the remainder of his neurologic examination findings were unremarkable. Cervical spine CT revealed C1-2 rotary subluxation.

The patient was admitted and placed in halo traction (5 pounds) with realignment of his vertebral bodies. A halo vest was applied after 3 days of cervical traction, and he was discharged after several days of physical therapy to assist in regaining mobility with the halo vest in place. After 10 weeks of cervical spine immobilization, lateral cervical radiographs demonstrated excellent cervical alignment. The halo vest was replaced with a soft collar that the patient wore for approximately 4 weeks. Activity restrictions, except for full contact sports, were lifted 9 months after the initial procedure.

Case 3. A 7-year-old boy presented with a persistent perforation of the left tympanic membrane associated with an attic retraction pocket in which there was retained squamous debris. He underwent a postauricular canal wall–down tympanomastoidectomy and type III tympanoplasty. After operation, he did well, and he was discharged the morning after surgery. During routine follow-up evaluation 1 week after surgery, he complained that his neck had been "stiff" since surgery. His head was rotated to the right and flexed to the left, and movement to the left past the midline was uncomfortable. There were no focal neurologic deficits.

A 3-dimensional cervical spine CT scan revealed C1-2 rotary subluxation. The C1-2 rotary subluxation was reduced manually, and his cervical spine was immobilized with a Miami J collar. He was followed on a weekly basis for the next 3 weeks with serial plain films of the cervical spine. After 12 weeks of cervical spine immobilization, he was prescribed use of a Philadelphia collar for 2 weeks, followed by use of a soft cervical collar that was gradually discontinued. His activity restrictions were lifted 5 months after his tympanomastoidectomy.

Case 4. An 8-year-old girl presented with a 1-day history of an edematous, draining left ear. Her history was remarkable for a left infratemporal fossa rhabdomyosarcoma that was treated initially with surgery and then with radiotherapy and chemotherapy. Tumor recurrence had necessitated additional chemotherapy and a salvage operative procedure.

Her physical examination was remarkable for a left middle ear soft tissue mass, a small eschar in the left preauricular region, and soft tissue fullness of the left postauricular sulcus. Cranial nerve VII was grade 1 bilaterally. There were no other head or neck abnormalities. A biopsy was consistent with Ewing's sarcoma rather than rhabdomyosarcoma, and on MRI the disease was seen to extend through the left temporal bone to the carotid artery. The metastatic workup was negative. After prolonged discussion with the parents, the patient underwent a left near-total temporal bone resection, left cranial nerve VII dissection and rerouting, left temporal craniotomy with dural resection and repair, and rectus abdominis free-flap reconstruction.

On postoperative day 5, she began to complain of discomfort with leftward head turning. A cervical spine CT revealed 28° of subluxation of C1-2, and the patient was placed in a halo crown with 5 pounds of traction. The following day, cervical spine films showed good bony alignment, and she was placed in

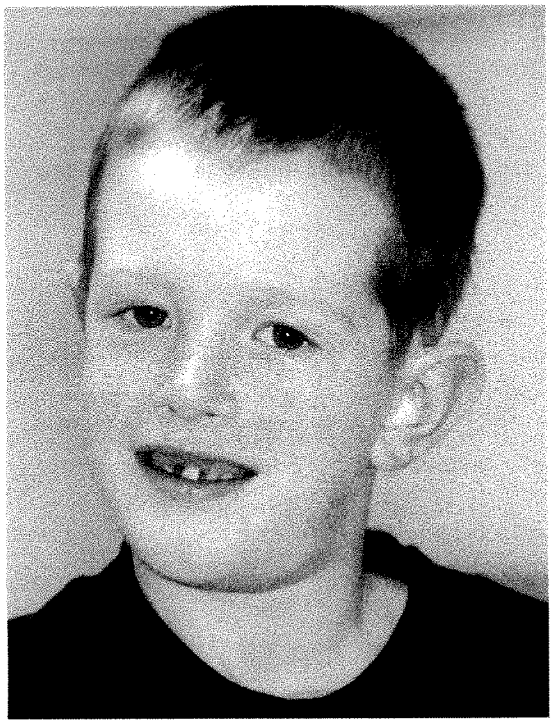

Fig 2. (Case 3) Child with "cock-robin" deformity. Head is tilted to, and chin is rotated away from, side of subluxation.

a halo vest. She remained immobilized in the vest for 4 weeks and used a Philadelphia collar for an additional 6 weeks. The Philadelphia collar was replaced with a soft cervical collar that was used for 2 additional weeks and then gradually discontinued.

DISCUSSION

Nontraumatic atlantoaxial rotary subluxation is an uncommon condition. Although its presentation varies depending on the degree of subluxation, the most common symptom is neck stiffness and pain on attempted motion. Neurologic signs are infrequent, but can include hyperreflexia and bladder dysfunction. Physical examination will reveal torticollis with the head tilted to, and the chin rotated away from, the side of subluxation ("cock-robin" deformity; Fig 2). The spinous process of the second cervical vertebra may be palpably displaced contralateral to the side of subluxation (Sudek's sign), and the anterior arch of the atlas occasionally can be seen or palpated in the nasopharynx, producing a mass effect and hyponasality.

The atlantoaxial joint is a complex articulation with 3 diarthrodial joints: 2 lateral facets and a median atlantodental articulation. Anterior atlantoaxial subluxation with posterior movement of the dens is normally prevented by the transverse cruciform and odontoid ligaments.[3] However, rotation on the axis is limited only by the alar ligaments.[4] Fielding and Hawkins[5] describe 4 types of atlantoaxial rotary subluxation. Type I, the most common, consists of rotation with minimal anterior subluxation. Type II involves rotation and less than 5 mm of anterior subluxation. Type III has more than 5 mm of anterior subluxation. With type IV, there is rotation with posterior subluxation.

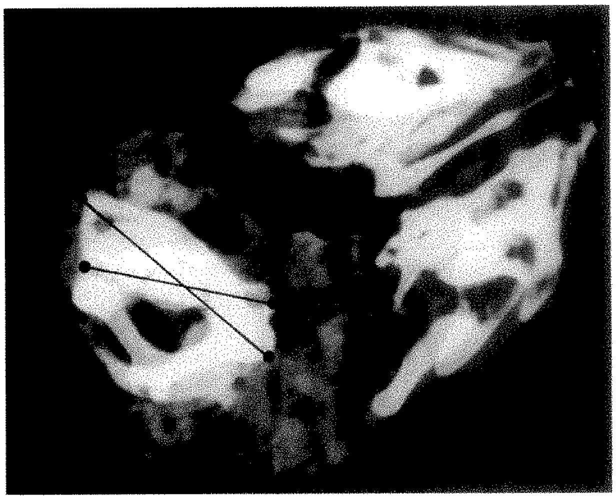

Fig 3. Superimposed axial computed tomography scans at levels of C1 and C2, demonstrating rotary subluxation. Transverse foramina of adjacent vertebrae are rotated out of alignment by 22° (lines). (Reproduced from Manaligod JM, Bauman NM, Menezes AH, Smith RJH. Cervical vertebral anomalies in patients with anomalies of the head and neck. Ann Otol Rhinol Laryngol 1999;108:925-33.)

Atlantoaxial rotary subluxation in the postoperative pediatric population is well documented. Procedures especially prone to induce this condition include those involving the pharynx, tonsils, and mastoid bone. Spasm of the deep cervical muscles and laxity of the supporting ligaments secondary to reactive inflammation may be contributory,[4] as demonstrated by Parke et al[6] in their study of vascular and lymphatic anatomy in 6 human perinatal cadavers. These authors identified a plexus of pharyngeal vertebral veins that permitted retrograde blood flow from the posterior superior nasopharynx and lateral pharyngeal recesses to the venous plexus surrounding the dens and atlantoaxial joints and ligaments. The pharyngeal lymphatics also drain into the venous plexus surrounding the odontoid complex, providing another avenue for inflammatory spread.[6]

Diagnosis of NAARS with plain radiographs depends on recognizing an asymmetry of the dens and surfaces of the lateral facet joints between C1 and C2 on an anteroposterior projection.[3] An increase in the atlantodens interval also may be seen on the lateral view. However, in persons with a normal cervical spine, rotation of the neck will induce atlantoaxial asymmetry, underscoring the need to properly position the patient during the imaging study.[1,3] A diagnosis of NAARS should not be made unless there is a persistent asymmetric relationship of the dens to the articular masses of the atlas that is not correctable with positioning.[1] Computed tomography is the imaging study of choice; rotary subluxation is most easily detected by superimposing images of C1 and C2 from a standard neck CT while aligning the mandible (Fig 3). Lack of alignment of the transverse foramina of the atlas and axis indicates rotary subluxation of the atlantoaxial complex.

Without proper recognition and treatment, untoward complications of NAARS can occur.[1] Management of postinflammatory NAARS requires treatment of the underlying cause and reduction of the subluxation. Less severe forms of NAARS may respond to conservative treatment, such as muscle relaxants and a hard cervical collar, although skull traction usually is necessary to reduce the dislocation. Once reduction is confirmed via radiography, a halo vest is applied. The halo vest typically is worn for 6 to 12 weeks. If the joint remains unstable or the subluxation is irreducible, surgical fusion may be necessary. With appropriate treatment, the prognosis of NAARS is excellent, but without treatment, torticollis will persist, with possible development of facial asymmetry (ipsilateral flattening).

CONCLUSIONS

1. Nontraumatic atlantoaxial rotary subluxation is an uncommon complication that follows pediatric head and neck infectious processes or operative procedures, especially procedures in which intraoperative rotation of the neck past 45° is required or the operative time is long.
2. The most obvious clinical finding is the "cock-robin" deformity, in which the head is tilted to, and the chin is rotated away from, the affected side.
3. Radiographic confirmation is difficult with plain films of the cervical spine, and is best achieved with a cervical spine CT scan.
4. Treatment is necessary to prevent long-term sequelae.

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EUSTACHIAN TUBE FUNCTION IN CHILDREN AFTER INSERTION OF VENTILATION TUBES

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This study was performed to assess the effect of the insertion of ventilation tubes and the subsequent aeration of the middle ear on eustachian tube (ET) function in children. Manometric ET function tests were performed repeatedly for 3 months after the placement of ventilation tubes in 83 children with otitis media with effusion (OME). Opening and closing pressures (passive tubal function) and active tubal function were measured. Analysis of the results showed a significant increase in opening pressure over time, whereas the closing pressure did not change. The active tubal function did not change and remained at the same poor level. Therefore, the opening pressure and closing pressure and, especially, the poor active tubal function, were more likely to be a causal factor of OME than a result. Certain children may have poor intrinsic ET function that makes them more susceptible to OME.

KEY WORDS — eustachian tube, otitis media with effusion, ventilation tube.

INTRODUCTION

Otitis media with effusion (OME) is a common disease during childhood. Nearly every child will have at least 1 episode of OME before the age of 4 years.[1] As a result of this high incidence, OME is the most frequent indication for surgery in young children.[2] Different surgical procedures, including myringotomy, insertion of ventilation tubes, adenoidectomy, or combinations thereof, are used for the treatment of OME.[3,4]

The pathogenesis of OME is multifactorial, but a negative middle ear pressure,[5-8] immunologic abnormalities,[9,10] or a combination of these factors is assumed to play a central role in the pathogenesis of OME.

The exact mechanism responsible for persistent negative middle ear pressure still remains unclear. Middle ear pressure is influenced by the diffusion of gases between the middle ear cavity and the surrounding tissues and by ventilation of the middle ear through the eustachian tube (ET). Impaired gas diffusion is speculated to be an important factor in the development of persistent negative middle ear pressure.[7,11] Normally, equilibration of negative middle ear pressure is achieved by periodic active opening of the ET. Impaired ventilation of the middle ear caused by functional obstruction of the ET[5,6] or closing failure combined with habitual sniffing[8] is assumed to cause persistent negative middle ear pressure. However, it is not clear whether this ET dysfunction is a cause or result of the effusion in OME. The ET function may deteriorate as a result of the presence of effusion in the middle ear cavity, or ET function may be intrinsically poorer in some children, who, as a result, develop persistent OME, whereas other children with better ET function do not develop OME or develop only short-term OME.

Many other factors described as prognostic or pathogenic for OME[12-15] probably influence the occurrence of OME through their effect on ET function, the diffusion of gases, or the immunologic response.

The insertion of ventilation tubes is assumed to be a symptomatic treatment. Ventilation tubes take over the function of the ET by resolving the effusion and ventilating the middle ear. However, besides being a substitute for ET function, the insertion of ventilation tubes may affect ET function as well. The aim of this study was to assess the effect of the insertion of ventilation tubes and the subsequent aeration of the middle ear on ET function in children with OME.

MATERIALS AND METHODS

The study population comprised 83 children (38 boys and 45 girls) with a mean age of 6.3 years (range, 1.7 to 12.1 years). All children were treated with insertion of ventilation tubes at the Department of Otorhinolaryngology of the University Medical Center or the Canisius Wilhelmina Hospital in Nijmegen because of persistent OME. In total, 138 ears were investigated (71 left ears and 67 right ears). On average, manometric ET function tests (forced-response

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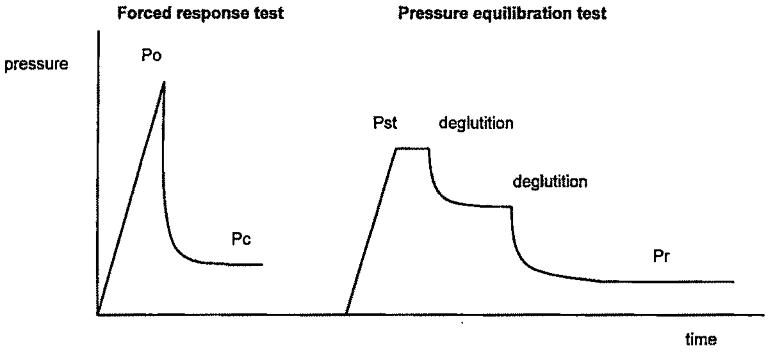

Fig 1. Example of eustachian tube measurement. Po — opening pressure, Pc — closing pressure, Pst — starting pressure, Pr — residual pressure.

test and pressure equilibration test) were performed 2 hours, 1.5 weeks, and 13 weeks after the operation. Children with syndromes that may affect middle ear function (eg, Down's syndrome, cleft palate) were excluded. Parental consent was obtained in all cases.

Equipment. A recently calibrated TYMP87 electroacoustic impedance bridge (Rexton Danplex A/S, Copenhagen, Denmark) was used for the measurements. The pressure applied by the pump ranged from 600 daPa to –600 daPa, with a pump rate of 50 daPa/s.

Procedure. Before surgery, all the parents filled in a questionnaire about their child's otorhinolaryngological history. Under general anesthesia, all the children underwent unilateral or bilateral myringotomy with aspiration of the effusion when present. Subsequently, ventilation tubes were inserted.

The ET function was assessed 2 hours after operation. An example of an ET function measurement is shown in Fig 1. Passive ET function was assessed by measuring the opening pressure (Po) and the closing pressure (Pc), ie, the forced-response test. The Po was the pressure at which the ET opened spontaneously, and the Pc was the residual pressure after passive opening. In a previous study, the results of the forced response test showed a downward shift with repeated measurement. Therefore, Po and Pc were recorded 3 times during each postoperative measurement, hereinafter referred to as a session, and the means of the 3 Po and Pc results were used for analysis.[16]

Active ET function was assessed by measuring the children's ability to equilibrate positive and negative pressures (pressure equilibration test). A positive or negative pressure was applied to the middle ear, and the effect of repetitive deglutitions (the children were asked to swallow some lemonade) on this pressure was recorded. The pressure equilibration test was performed only once during each session, since the categorized results of the test showed good reproducibility.[16] The residual pressure (Pr) after several deglutitions was recorded. On the basis of the Pr, each subject was classified into 1 of 4 tubal function groups according to Elner et al.[17] The subjects in group I were able to equilibrate positive and negative pressures completely (Pr less than 10 daPa and greater than –10 daPa, respectively). Group II subjects could equilibrate positive and negative pressures partially (Pr greater than 10 daPa and less than –10 daPa respectively). Group III subjects could equilibrate positive pressure completely or partially, but were not able to equilibrate negative pressure. The subjects in group IV could not equilibrate positive or negative pressures. The measurements were performed with starting pressures of 200 and –200 daPa, respectively. In some subjects, a starting pressure of 200 daPa could not be applied, because the ET opened spontaneously before 200 daPa was reached. In these subjects, the test was performed with starting pressures of 100 and –100 daPa in all 3 sessions. The ET function tests are described in more detail elsewhere.[16]

Statistical Analysis. Univariate analyses (SAS) were performed on the data from the ears that were measured first. Only one ear of each child was used for univariate analyses, because the results of the left and right ears were highly correlated. Differences in mean Po and Pc over time were tested by a paired *t*-test, and differences in tubal function group over time were tested by McNemar's test.

A χ^2 test and Student's *t*-test were used to study the differences in active and passive tubal function between different subgroups. For each comparison, the children were divided into 2 subgroups on the basis of 1 of the following criteria: previous adenoidectomy, previous treatment with ventilation tubes, ≥4 annual episodes of upper respiratory tract infection, and the presence of effusion in the middle ear at the time of surgery.

In order to adjust for comparison of multiple test sessions, we also analyzed Po and Pc from all ears after logarithmic transformation using a linear mixed model (SAS procedure mixed). The effect of order of measurement (ie, whether the left ear or the right ear was measured first) was also studied in this model. We used time (3 levels) and order of measure-

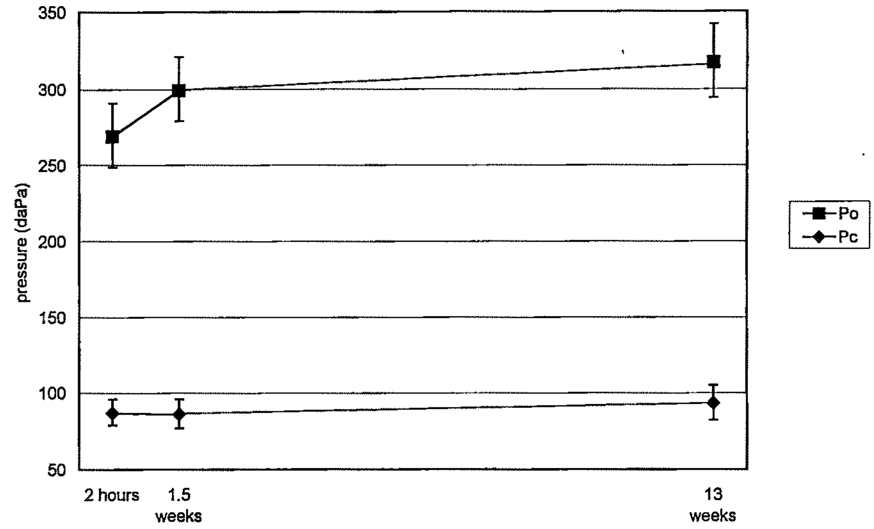

Fig 2. Mean opening and closing pressures (±2 SEM) at 2 hours, 1.5 weeks, and 13 weeks, based on results of linear mixed model.

ment (2 levels) as additive fixed effects. An unstructured covariance matrix was used. Some children's ET did not open even when the maximum pressure (ie, 600 daPa) was applied. To be able to include these ears in the linear mixed model, we estimated the Po of these ears to be 650 daPa, on the basis of the variance of all Po. The corresponding Pc remained missing, because no reliable estimation of Pc could be made.

The ears in tubal function groups I and II were omitted from all of the analyses, because these groups contained no or only a few ears. Data from the children with missing values at 1 of the sessions due to otorrhea, obstruction or early extrusion of the ventilation tube, or poor cooperation of the child were included in the analyses, because the occurrence of these missing values was assumed to be random and because the results of these children corresponded with the results of the children without missing values.

RESULTS

Forced Response Test. Figure 2 shows the mean Po and Pc ± 2 SEM of all the ears at 2 hours, 1.5 weeks, and 13 weeks based on the results of the linear mixed model. Both univariate analysis and the linear mixed model showed a significant increase in Po over time (p = .003, linear mixed model). The increase in Po between the first and the second sessions was more pronounced (30 daPa in 1.5 weeks, p = .01) than the increase between the second and third sessions (18 daPa in 11.5 weeks, p = .06). The order of measurement (ie, whether the left or the right ear was measured first) did not affect the Po (p = .86). We found no effect of time or order of measurement on Pc (p = .34 and p = .24, respectively).

Pressure Equilibration Test. Figure 3 shows the distribution over the tubal function groups at 2 hours, 1.5 weeks, and 13 weeks. Only a few children were able to equilibrate negative pressure (4%, 2%, and 3% at the 3 sessions, respectively), and none of these children were able to equilibrate negative pressure at all 3 sessions. Most of the children had poor ET function, ie, group III or IV tubal function. No significant change in tubal function group was found on McNemar's test during the 3-month follow-up period (p ≥ .2), but there was considerable intraindi-

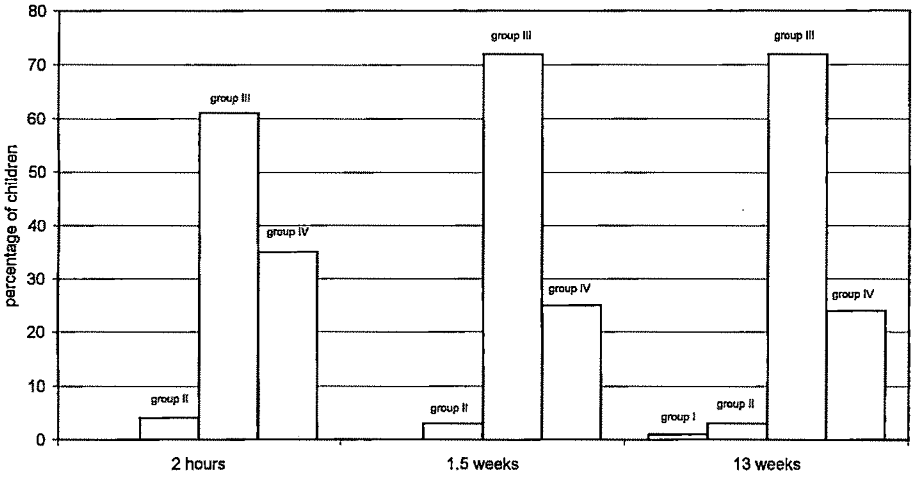

Fig 3. Active tubal function at 2 hours, 1.5 weeks, and 13 weeks.

ACTIVE AND PASSIVE TUBAL FUNCTION OF EACH SUBGROUP AT FIRST SESSION

Subgroups	*n*	*Opening Pressure (daPa; mean ± SD)*	*Closing Pressure (daPa; mean ± SD)*	*Group III (%)*	*Group IV (%)*
Previous adenoidectomy					
Yes	43	282 ± 120	114 ± 63	63	37
No	39	273 ± 116	102 ± 60	63	37
Previous ventilation tubes					
Yes	42	287 ± 126	109 ± 68	59	41
No	40	268 ± 109	108 ± 54	68	32
Upper respiratory tract infections					
≥4/y	44	270 ± 102	109 ± 54	68	32
<4/y	38	286 ± 135	108 ± 70	57	43
Effusion					
Present	47	302 ± 136*	125 ± 68*	54*	46*
Not present	27	234 ± 74*	78 ± 44*	77*	23*

*Statistically significant difference.

vidual variability over time. An individual's ET function group was found to fluctuate over time.

Associations. The Po and Pc were found to be significantly higher in tubal function group IV than in group III at all 3 sessions (all p ≤ .005). Two hours after operation, the Po was 79 daPa higher in group IV (95% confidence interval [CI], 26 to 132 daPa), whereas the Pc was 46 daPa higher (95% CI, 19 to 73 daPa). At 1.5 and 13 weeks, these differences were even more pronounced.

The Table shows the results of the active and passive tubal function of the different subgroups at 2 hours after operation. Univariate analysis did not show any significant differences in active or passive tubal function between children with and children without previous adenoidectomy, ventilation tubes, or upper respiratory tract infections at any of the 3 sessions. However, the presence of effusion at the time of surgery was found to affect active and passive tubal function. In 27 of the 83 children, the effusion in the ear that was used for univariate analysis had resolved before the insertion of ventilation tubes. In these ears, no effusion was seen during surgery. In 47 ears, effusion was present, and in 9 ears, this information was missing. The ears with and without effusion were compared. At 2 hours after operation, the mean Po was found to be 68 daPa higher in the ears with effusion (95% CI, 11 to 124 daPa), whereas the Pc was 47 daPa higher (95% CI, 17 to 76 daPa). Active tubal function was found to be poorer in the ears with effusion: 46% of the ears with effusion had group IV tubal function, compared to 23% of the ears without effusion (p = .003). At 1.5 and 13 weeks, these differences in Po, Pc, and tubal function group between the ears with and without effusion at the time of surgery still existed, but they were smaller and were no longer statistically significant.

DISCUSSION

The insertion of ventilation tubes had no effect over time on tubal function group or Pc, but it did result in a significant increase in Po. If it were the case that Po and Pc increase and active tubal function deteriorates as a result of effusion in the middle ear (ie, that ET dysfunction is a result of the effusion), then we would have found a decrease in the Po and Pc and an improvement in active tubal function when the effusion is resolved by inserting ventilation tubes. However, Po and Pc did not decrease (Po even increased), and active tubal function remained at the same poor level. Therefore, the Po and Pc and especially the poor active tubal function in these children were more likely to be a cause than a result of the effusion, although it cannot be ruled out that effusion in the middle ear may also affect ET function. Apparently, some children have poor intrinsic ET function, which makes them more susceptible to OME and maybe to other middle ear diseases as well.

The Po is assumed to reflect the total closing forces (ie, the luminal and extraluminal forces) of the ET. The Pc is assumed to represent the extraluminal forces (pressure of the cartilage and other surrounding tissues, etc). The difference between Po and Pc is assumed to represent the luminal forces (mucosal factors, surface tension, viscosity of secretions, etc).[18-20] Because Po increased and Pc remained constant, it may be inferred that the insertion of ventilation tubes caused an increase in the luminal forces. It has been shown that the insertion of ventilation tubes caused a change in the middle ear gas composition.[21] Several authors found that this change in middle ear gas composition in vivo or in vitro caused changes in the middle ear epithelium.[22-25] Changes in the middle ear epithelium and/or possibly subsequent changes in the secretion of mucus and mucoadhesive substances may result in higher luminal forces and thus higher Po. This hypothesis suggests that the insertion of ventilation tubes may even have an adverse effect on ET function, because higher Po and Pc were associated with poorer active tubal function.

The Po and Pc were significantly higher and active tubal function was significantly poorer in the ears with effusion on the day of surgery. In both subgroups, the tubal function group and Pc did not change, whereas Po increased throughout the follow-up period. For the reasons discussed above, the ears in which no effusion was found at the time of tube insertion may have had better intrinsic ET function than the ears in which effusion was present at the time of surgery. It may even be hypothesized that ET function also affects the degree of persistence of the effusion, besides influencing the occurrence of effusion.

The literature on the effect of the insertion of ventilation tubes and the subsequent aeration of the middle ear on ET function is limited. Most studies did not find significant changes in active or passive tubal function over time.[26-29] However, most of these studies comprised small study populations, used different time intervals, and studied only active or passive tubal function. Recently, Bunne et al[30] reported that Po increased significantly over time, whereas active tubal function did not change. Although they also reported a significant increase in the mean Pc, the median Pc remained fairly stable. Apparently, their results were positively skewed. Thus, our results correspond with theirs.

CONCLUSION

Poor ET function is more likely to be a causal factor in OME than a result, because Po and Pc did not decrease (Po even increased), and active tubal function remained at the same poor level, after the insertion of ventilation tubes and subsequent drainage of the effusion. Certain children may have poor intrinsic ET function that makes them more susceptible to OME and maybe to other middle ear diseases as well. The increase in luminal forces after the insertion of ventilation tubes, believed to be caused by mucosal changes due to altered middle ear gas composition, may even have an adverse effect on passive ET function. These findings indicate that ET function tests may be a useful diagnostic tool for discriminating between different degrees of middle ear disease. In addition, treatment strategies that potentially affect ET function, such as adenoidectomy, may be more effective in children with poor ET function than in those with better ET function. These prospects for the application of ET function tests in the diagnosis and management of OME should be investigated in further studies.

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MORPHOMETRIC ANALYSIS OF AGE-RELATED CHANGES IN THE HUMAN BASILAR MEMBRANE

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The histopathologic correlates of presbycusis suggest several categories, including degeneration of sensory cells, neurons, or the stria vascularis. Lack of clear-cut histopathologic changes in some cases has suggested an "indeterminate category"; however, several studies have suggested that a disorder of the basilar membrane (BM) may underlie indeterminate presbycusis. The objective of the present study was to quantify age-related changes in the human BM and correlate them with audiometric patterns. Under high-resolution light microscopy, BM thickness was calculated, and the number of tympanic mesothelial cells (TMCs) lining the BM was counted, at 4 cochlear locations in 92 temporal bones. The control group (n = 80) included subjects from 10 decades of age with normal hearing and/or histopathologic findings. The indeterminate group (n = 12) consisted of elderly patients (ages 64 to 91 years) with hearing loss and no apparent histopathologic changes. Age-related BM thickening was seen in both groups, but only in the most basal cochlear region. The BM thickness in the indeterminate group was not significantly different from that of age-matched controls. Counts of TMCs showed age-related decreases in all cochlear regions in both groups; however, TMC counts in the indeterminate group were not different from those of age-matched controls. The results suggest that BM histopathology is not a common cause of presbycusis. Although age-related BM thickening, seen in both groups, could contribute to hearing loss, the extreme basal region, to which the thickening was confined, is not tested in routine audiometry.

KEY WORDS — basilar membrane, cochlea, histopathology, human, inner ear, presbycusis, tympanic mesothelial cell.

INTRODUCTION

Presbycusis is defined as hearing loss that is coincident with aging. It is characterized by deafness that is slowly progressive and bilaterally symmetric, and has its onset in the 3rd or 4th decade of life. The cause of presbycusis is undoubtedly multifactorial. Histopathologic studies of human temporal bones suggest that there may be several categories of presbycusis, including "sensory," "neural," and "metabolic" ("strial") presbycusis.[1,2] While each of these types occurs often enough to warrant their individual classification, the majority of patients with presbycusis have a combination of disorders, or mixed presbycusis.

In sensory presbycusis, the predominant histopathologic correlate of hearing loss is the loss of hair cells. It is characterized by hair cell loss beginning at the basal end of the cochlea and extending several millimeters in length. There is a gradual loss of outer hair cell density at the base and apex with age; there is a similar but less marked pattern for inner hair cell loss.[3] Most lesions are within the 8- to 12-mm region of the aging cochlea, corresponding to the 4-kHz frequency. The audiometric findings in sensory presbycusis demonstrate an abrupt, high-frequency sensorineural hearing loss (SNHL) with slow, symmetric bilateral progression beginning in middle age.

In neural presbycusis, the principal histopathologic correlate of hearing loss is loss of cochlear neurons. The audiogram demonstrates a moderate slope of pure tone thresholds, worse in the high frequencies, and a decrease in speech discrimination scores (phonemic regression). The loss of cochlear neurons begins at an early age and continues throughout life; usually, there is no appreciable effect on pure tone thresholds until the number of neurons falls below roughly 90% of normal.[4]

In metabolic presbycusis, the principal histopathologic finding is atrophy of the stria vascularis. It is characterized by hearing loss that has an insidious onset in the 3rd to the 6th decades. The audiogram is typified by flat or slightly descending pure tone thresholds with excellent word discrimination scores. It commonly affects several family members. The histopathologic findings include patchy atrophy of the stria vascularis in the middle and apical turns of the

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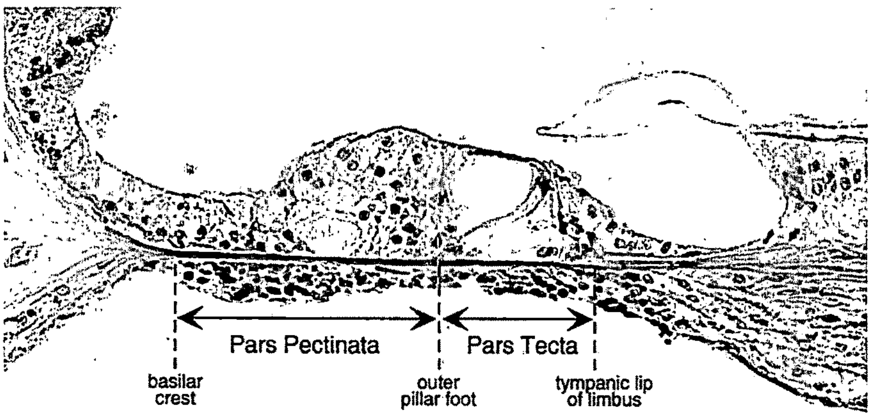


Fig 1. Anatomic divisions of basilar membrane (BM). Outer pillar cell divides BM into 2 regions. Pars pectinata was defined as that region between foot of outer pillar cell and basilar crest of spiral ligament. Pars tecta was defined as region between outer pillar cell and tympanic lip of spiral limbus.

cochlea; there may be partial or complete loss of strial cells, sometimes with cystic formation and basophilic deposits within the stria.[5] It is believed that the atrophy of strial tissue causes dysfunction in the processes responsible for ion homeostasis in the cochlea.

"Indeterminate presbycusis" is a diagnosis of exclusion, made when there is no significant loss of hair cells, sensory cells, or strial tissue to reasonably correlate with the hearing loss.[6] There are numerous possible causes for indeterminate presbycusis, including the possibility that the pathological changes responsible are submicroscopic. However, most speculation has centered on the spiral ligament and basilar membrane (BM), because there is evidence for histopathologic changes in these structures, at least in some temporal bones from aged individuals.[3,7-10]

The idea that histopathologic changes in the BM might be important in SNHL can be traced at least as far back as 1919, when Mayer[11] observed BM thickening and hyaline degeneration. Crowe et al[12] also reported hyalinization and deposition of calcium salts in the basalmost BM to be more common in individuals with descending audiometric findings than in persons with normal ears. In addition, they suggested that while partial atrophy of basal turn neurons was a prominent lesion in many ears with descending audiograms, the degree of nerve atrophy was sometimes insufficient to explain the hearing loss.[12] In 1970, Nomura[8] found lipid deposits on the BM along the filamentous structure of the pars pectinata in 9 of 20 patients, and suggested they were responsible for some of the age-related hearing loss. The lipids, composed of neutral fat and cholesterol, were deposited in the most basal 10 mm, in which the organ of Corti could be either present or missing.

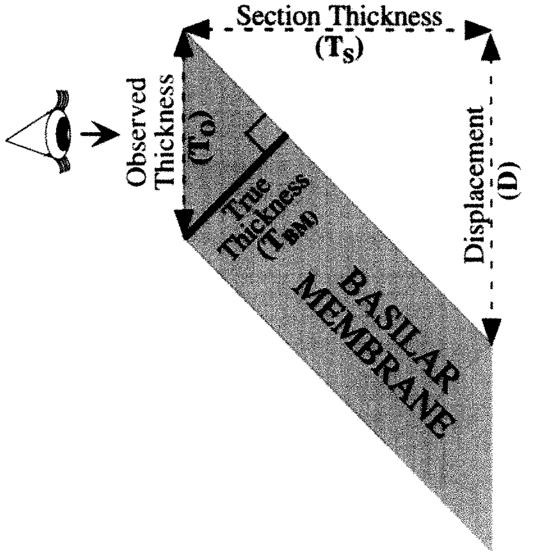
In 1979, Nadol[7] examined the cochlear ultrastructure in an 81-year-old with progressive bilateral hearing loss. In addition to severe atrophic changes in the organ of Corti, this individual had BM thickening in the basal turn, associated with an increased number of fibrils and an accumulation of amorphous osmiophilic material in the BM. Another electron microscopic study of the aged ear by Ishii et al[9] also showed thickening of the BM in the basal turn of the rat, accompanied by an increase in the homogeneous ground substance. Shimada et al,[13] in a study of age-related changes in the dog cochlea, noted that BM thickening first became evident at 3 months of age. Furthermore, they noted that the change was prominent at the basal region of the cochlea, and that in the advanced stage, the thickened BM was hyalinized and positive for periodic acid–Schiff staining.[13]

In the present study, we set out to rigorously test the hypothesis that BM histopathology can contribute to age-related hearing loss in human patients. In a single-blind study, data on the BM were obtained from a set of 92 temporal bones by means of high-resolution Nomarski optics. The set of bones included a control group of normal-hearing individuals, spanning 10 decades of age, and an indeterminate group of aged individuals with significant hearing loss for which no adequate correlation could be made between the audiometric findings and light microscopic histopathologic findings in the hair cells, neurons, or stria vascularis.

METHODS

This study consisted of a systematic evaluation of 92 temporal bones in the collection at the Massachusetts Eye and Ear Infirmary. The inclusion criteria for the control group (n = 80) were normal audiometric findings or an absence of significant histopathologic changes in the hair cells, neurons, and stria vascularis. Representative cases were selected from the 1st through the 10th decades. For the "indeterminate" group (n = 12), the criterion for inclusion was SNHL without correlative histopathologic changes in the hair cells, neurons, or stria vascularis.

The temporal bones had been prepared for light microscopic study by standard techniques involving fixation, decalcification, celloidin-embedding, 20-μm horizontal sectioning, and hematoxylin and eosin



$$T_{BM} = T_O * (\sin (90 - \arctan (D/T_S)))$$

Fig 2. Cross-sectional diagram of BM. At midpoint of pars pectinata or pars tecta, observed height of BM was measured at most superior focal plane (TBM). After selection of point in one focal plane, its corresponding point was identified in deepest portion of BM (z-axis) by means of fine focus control. Distance between two planes was section thickness, Ts. Y was vertical distance of travel required to reach from one plane to last. Both Ts and Y were measured by Neurolucida. True height or actual thickness of BM was then calculated according to formula: Actual thickness = TBM × (sin[90 − arctan(Y/Ts)]).

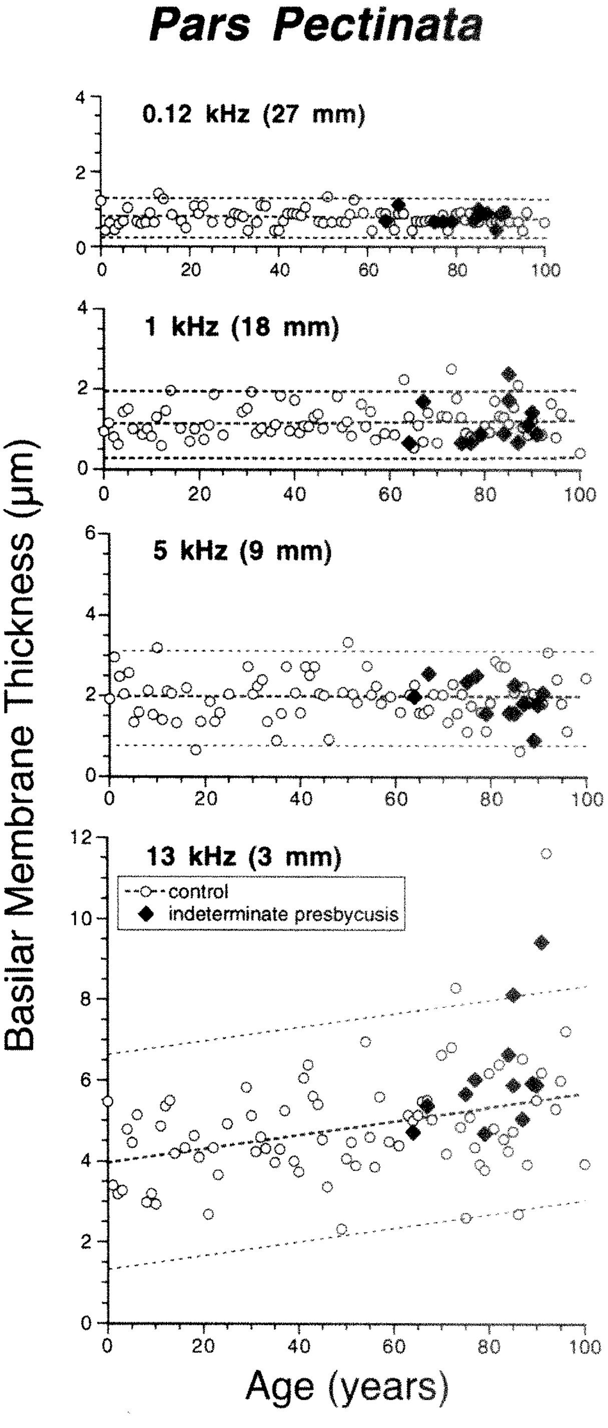


Fig 3. BM thickness at pars pectinata versus age. There was significant (Pearson $p < .0001$) age-related thickening of BM, limited to basal cochlear region. There was linear increase in BM thickening, and increased variability of thickening with age. Mean thickness was initially 3.86 µm, and increased at rate of 0.021 × age (years). There was no significant difference in thickness between control and indeterminate presbycusis groups.

staining of every 10th section.[14] Using Nomarski optics and oil-immersion objectives, we made blinded randomized measurements at 4 different cochlear locations in each case: the hook portion (on average, 3 mm from the round window) and the lower basal (9 mm), midmodiolar (18 mm), and apical (27 mm) regions of the cochlea. At each location, 3 data points were collected (Fig 1): 1) BM thickness at the midpoint of the pars tecta, 2) BM thickness at the midpoint of the pars pectinata, and 3) the total number of tympanic mesothelial cells (TMCs) lining the undersurface of the BM: only cell nuclei were counted.

All BM measurements were made with computer-aided anatomic reconstruction software (Neurolucida, MicroBrightfield, Inc, Colchester, Vermont). To minimize measurement errors from tangential sectioning, each of the 4 cochlear locations, except the most basal, was chosen to be a midmodiolar section through a particular half-turn of the cochlea. The correction procedure for tangential-sectioning errors in the most basal ("hook") region is schematized in Fig 2. With high-power Nomarski optics and its inherent optical sectioning capabilities, the top and bottom of the histologic section can be easily identified and the section thickness measured, as can the vertical "displacement" of any point on the BM from the top to the bottom of the section. Then, the actual BM thickness can be calculated from the observed thick-

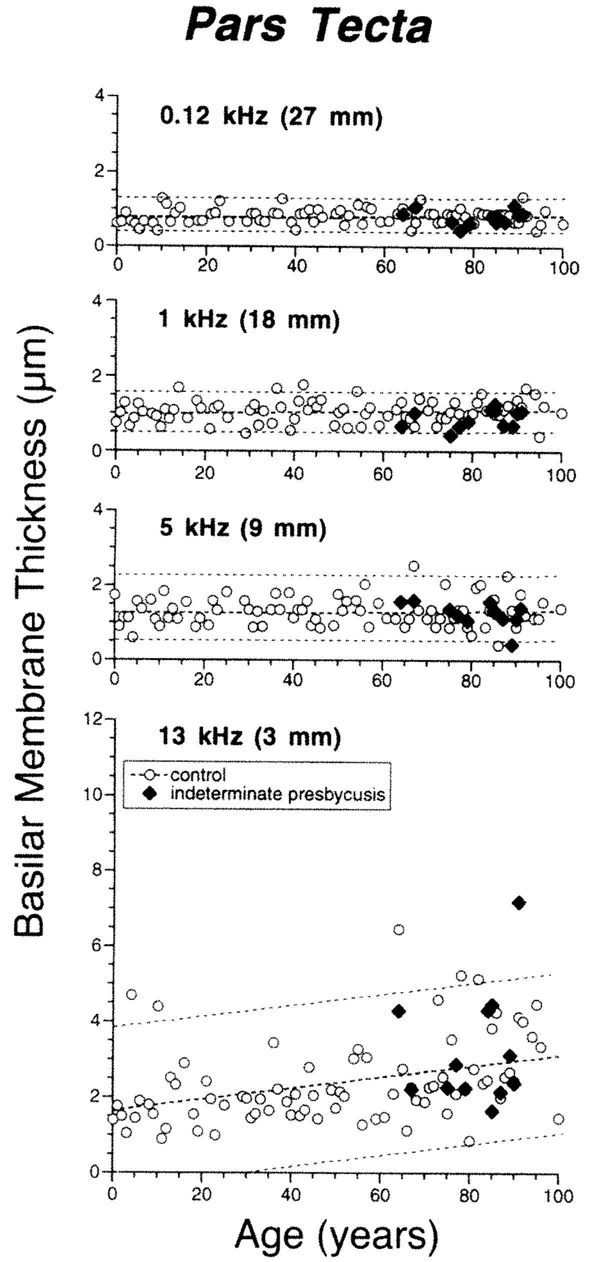


Fig 4. BM thickness at pars tecta versus age. There was significant (Pearson p < .001) age-related thickening of BM, limited to basal cochlear region. There was linear increase in BM thickening, and increased variability of thickening with age. Mean thickness was originally 1.67 μm, and increased at rate of 0.021 × age (years). There was no significant difference in thickness between control and indeterminate presbycusis groups.

ness according to the trigonometric formula shown in Fig 2.

RESULTS

Basilar Membrane Thickness. The BM morphometry revealed a significant age-related thickening for both the pars pectinata (Fig 3) and the pars tecta (Fig 4). For the control group of 80 ears, Pearson correlation coefficients showed that significant age-related thickening of the pars pectinata ($p < .0008$) and pars tecta ($p < .0045$) was limited to the most basal cochlear region. There was also greater variability in BM thickness in the hook among the older individuals. Analysis of variance suggested that the BM in the hook region increases in thickness linearly with age, according to the formulae Pars pectinata thickness (in microns) = 3.86 + [0.021 × age (in years)]; and Pars tecta thickness (in microns) = 1.67 + [0.014 × age (in years)].

Comparison of BM thickness in the indeterminate presbycusis group with that in the age-matched normal-hearing controls suggests there are no striking differences in either the pars pectinata (Fig 3) or the pars tecta (Fig 4). Statistical analysis confirmed those impressions. There were a few individuals in both groups for whom BM thickness in the most basal region fell more than 2 standard deviations from the age-matched mean.

Under Nomarski optics, it appeared that the BM thickening was associated with an increase in the deposition of fibrils, as well as a small amount of amorphous material. The staining patterns of the BM suggested that there was chemical heterogeneity in the thickening process: in some individuals, the BM was primarily basophilic, and in others, it was clearly eosinophilic. There was no systematic difference in the distribution of basophilia versus eosinophilia in the control group versus the indeterminate group.

Tympanic Mesothelial Cells. Figure 5 demonstrates that TMCs also show clear age-related changes: their numbers decrease steadily with age at all 4 cochlear locations analyzed. Pearson correlation coefficients showed that significant age-related decrease in TMCs occurred at the 4 cochlear locations analyzed ($p < .0001$). Again, however, there was no significant difference between the presbycusic group and the normal-hearing age-matched controls. In contrast to the BM thickness data, variability in TMC counts did not increase with increasing age. As others have noted, the number of TMCs increases steadily from base to apex in all age groups.[15,16] The average numbers of cells for the 3-, 9-, 18-, and 27-mm cochlear regions were 21, 51, 79, and 86 cells, respectively, for the control population, and 13, 45, 71, and 76 cells, respectively, for the indeterminate population.

DISCUSSION

Age-Related Changes in Normal-Hearing Individuals. The BM is a fundamental element of the mechanically tuned system for frequency analysis in the auditory periphery. It is made of radially oriented fibrils arranged into 1 or more discrete bundles, embedded in an amorphous ground substance, and separated from the cells of the organ of Corti by a discontinuous basement membrane.[17,18] This acellular layer of fibrils and ground substance is lined on the perilymphatic face by a relatively loosely packed layer of TMCs. The TMCs are arranged so that long, longitudinally oriented processes emanate from both sides of their centrally located cell body. The fibrillar arrangement differs within the 2 radial zones of the BM. In the pars tecta, where the entire BM is thinner, the fibrils tend to be organized into a single compact layer, whereas in the pars pectinata, they are more loosely organized into 2 or more strata separated by ground substance.[9,16,18] The total number of BM fibrils decreases dramatically from the base to the apex, with 10-fold more fibrils present in the base than in the apex; correspondingly, the proportion of BM occupied by fibrils versus ground substance also decreases from base to apex.[16] It is widely assumed that the longitudinal gradient in the density of BM fibrils must be an important contributor to the longitudinal gradient in BM stiffness, which, in turn, is critical to the BM's function as a mechanical frequency analyzer.

The chemical composition of these acellular components of the BM is not well understood. At the ultrastructural level, the diameter of the filaments ranges from 90 to 120 Å. When viewed in cross section, each filament is rectangular or diamond-shaped, with 4 or 5 subfibrils.[16] Slepecky et al[19] found that the filaments were not composed of type II, V, or IX collagen, and suggested that another type of collagen may be present. On the other hand, Mikuni et al[17] suggested that the filaments are neither collagen nor elastin fibrils; instead, they found in their study using transmission electron microscopy that the filaments were similar to the microfibrils of subcutaneous tissue.

The functional significance of the TMC layer is very poorly understood. As suggested by immunohistochemistry, it may not contribute to the mechanical properties of the system; rather, it may be involved in the production and/or maintenance of the fibrillar proteins and matrix materials for the otherwise acellular BM.[20,21] However, it may be that not all substances seen in the BM ground substance, eg, fibronectin, arise from TMCs.[20,22] It has also recently been shown that TMCs are rich in water channel proteins; however, the functional significance of this finding is not yet clear.[23,24] Whatever the functional role of TMCs, our findings of an apical-basal gradient in TMC density, at all ages, corroborate those reported for the rat[15] and cat.[16]

Our analysis of control, normal-hearing subjects showed significant age-related thickening of the BM

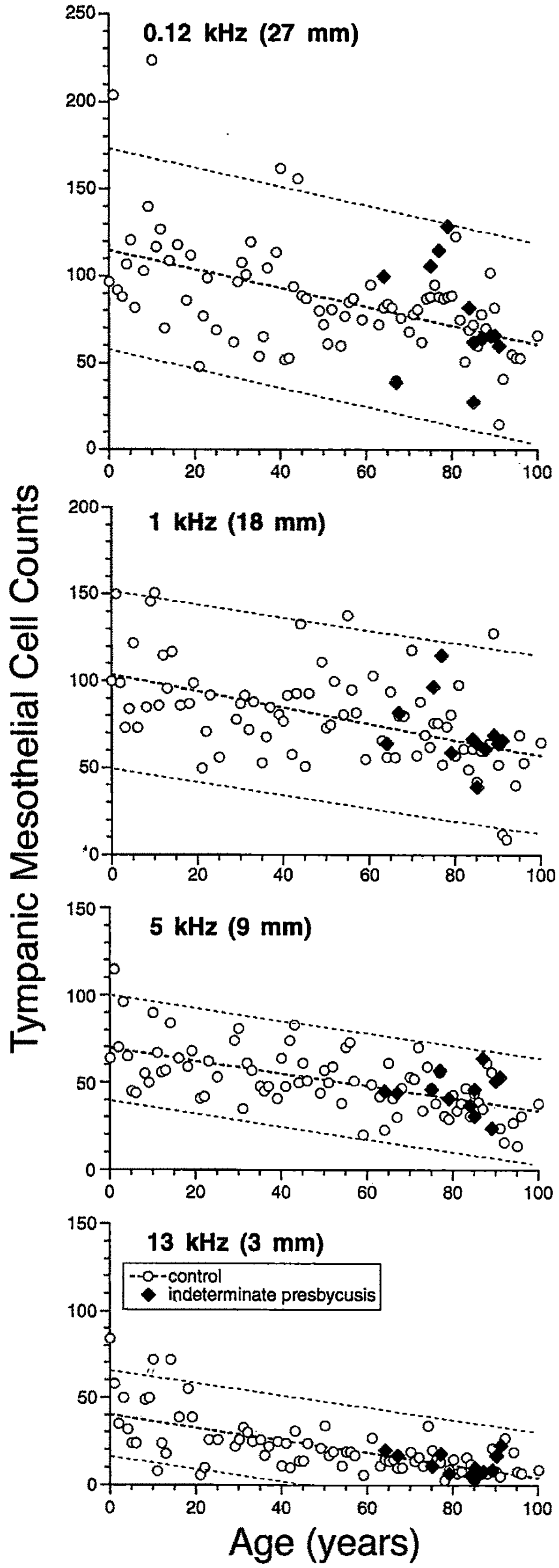


Fig 5. Tympanic mesothelial cells (number of nuclei) versus age. There was significant (Pearson $p < .0001$) age-related decrease in number of cells with age at all cochlear locations. There was no significant difference in cell counts between control and indeterminate presbycusis groups.

associated with age-related thinning of the TMC layer. The BM thickening was seen only in the most basal cochlear region analyzed, whereas the TMC layer thinning was seen in all regions investigated. Given that the TMC thinning was seen in normal-hearing subjects, the loss of TMCs must not have a significant effect on threshold sensitivity.

The BM thickness we measured included the entire acellular matrix comprising fibrils and ground substance, and our impression was that the aged BM was as densely packed with fibrils as the young BM. Thus, our analysis suggested an age-related increase in the number of BM fibrils in the hook region. As suggested by the regression lines in Figs 3 and 4, over a 100-year span there is an approximately 38% increase in pars pectinata thickness (4.0 to 5.5 µm) and an approximately 66% increase in pars tecta thickness (1.8 to 3.0 µm). There are several reasons why these changes may not have significant effects on the audiogram. First, these changes are limited to the region of the BM that, according to the cochlear frequency map for humans, is normally tuned to 13 kHz.[2] Since 13 kHz is beyond the highest frequency tested in routine audiometry, it is not surprising that normal hearing thresholds were seen despite the presence of this age-related change. Second, hearing levels are measured on a logarithmic scale, and each 20-dB loss corresponds to an additional 10-fold decrease in the amplitude of BM vibration. Therefore, it may be unrealistic to expect a less than 2-fold change in thickness to have profound effects on thresholds, even if thresholds were assessed at 13 kHz.

It is also possible that mechanical changes in the 13-kHz region could affect cochlear mechanics at more apical locations, ie, within the audiometrically tested range. Although a quantitative answer is difficult, a number of empirical observations suggest that any distant effects, ie, for frequencies an octave away, will be quite small. First, it has been shown in both animal models[25] and human subjects[26,27] that creating a large hole through the BM and organ of Corti in the extreme basal region has little effect on thresholds at audiometric frequencies (≤8.0 kHz). Similarly, total loss of the organ of Corti throughout large stretches of the basal turn (as seen after ototoxic antibiotics) does not lead to significant threshold elevations in cochlear regions more than 1 octave apical to the apical border of the lesion.[28,29] Nevertheless, in a study of distortion product otoacoustic emissions, Ramotowski and Kimberley[30] found evidence for a slight age-dependent increase in the traveling wave delay. Although they suggest that the effect might arise from an age-related *increase* in BM flexibility, the underlying cochlear mechanics are not well enough understood to rule out a contribution from increased BM thickening.

Contributions of Basilar Membrane Histopathology to Presbycusis. The present analysis showed no significant differences in BM histopathologic findings in the indeterminate presbycusis group versus the age-matched "normal" population; this outcome suggests that BM histopathology is not a major contributing factor in the causation of presbycusis. The criteria for inclusion in the normal control group were not strict, by necessity, given the nature of temporal bone studies; ie, not all individuals had audiometric data available, and for those without audiograms, the presence of histopathologic changes was not necessarily grounds for exclusion. For example, a 92-year-old in the normal group showing marked BM thickening and also had degeneration of the organ of Corti, the stria vascularis, and neural ganglion cells secondary to otosclerosis. The pathological findings were most severe at the basal end of the cochlea, but not severe or extensive enough to meet the existing (arbitrary) criteria for inclusion in the sensory, neural, or strial presbycusis groups.[1,2] Nevertheless, even if we ignore the somewhat arbitrary distinction made between the normal and indeterminate groups in the present study, the systematic and extensive morphological evaluations presented reveal that BM histopathologic changes are restricted to the extreme basal regions of the cochlea, and thus do not likely contribute to hearing loss at audiometric frequencies.

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GEOMETRIC CHARACTERIZATION OF THE LARYNGEAL CARTILAGE FRAMEWORK FOR THE PURPOSE OF BIOMECHANICAL MODELING

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Some new anatomic data on the laryngeal cartilage framework have been obtained for the biomechanical modeling of the larynx. This study attempted to define and measure some biomechanically important morphometric features of the laryngeal framework, including both the human and the canine laryngeal frameworks, because the canine larynx has been frequently used as an animal model in gross morphology and in physiological experiments. The larynges of 9 men, 7 women, and 9 dogs were harvested and dissected after death. Linear and angular geometric measurements on the thyroid cartilage, the cricoid cartilage, and the arytenoid cartilage were made with a digital caliper and a protractor, respectively. The results are useful for constructing quantitative biomechanical models of vocal fold vibration and posturing (abduction and adduction), eg, continuum mechanical models and finite-element models of the vocal folds.

KEY WORDS — anatomy, arytenoid cartilage, cricoid cartilage, laryngeal framework, morphometric measurement, thyroid cartilage.

INTRODUCTION

The laryngeal framework consists of the thyroid cartilage, the epiglottis, the cricoid cartilage, and the arytenoid cartilages. It houses the soft tissues of the vocal system, including the intrinsic laryngeal muscles, the true vocal folds, the false vocal folds, and the aryepiglottic folds. The morphology of the laryngeal framework determines the geometry and the actions of the laryngeal muscles, in particular, the vocal fold geometry, which is critical to the mechanics of phonation. For instance, adjustments in the prephonatory medial surface geometry of the vocal folds can lead to changes in vocal register, in periodicity of vocal fold vibration, and in ease of phonation.[1-3] Thus, anatomic data on the laryngeal framework are important for the biomechanical modeling of the larynx, including the simulation of vocal fold vibration, as well as vocal fold posturing (abduction and adduction). Moreover, electrophysiological and radiologic diagnostic studies (electromyography, computed tomography, and magnetic resonance imaging) and surgical treatments (eg, thyroplasty and arytenoid adduction) for laryngeal disorders demand precise anatomic knowledge of the laryngeal framework. For the human laryngeal cartilages, a variety of geometric measurements have been reported in the literature.[4-9] In particular, the work of Eckel et al[5] and Sprinzl et al[6] has been exhaustive and extremely relevant; it originally involved a total of 95 morphometric measurements on each of 53 normal human larynges (28 from male subjects and 25 from female subjects) not subjected to fixation and freshly dissected after death. As a follow-up, Sprinzl et al[6] reported 28 similar measurements on as many as 98 larynges (52 from male subjects and 46 from female subjects). Relevant measurements have also been reported on whole-organ horizontal and frontal sections of deep-frozen larynges.[7-9] However, many of these previously reported measurements were not ideal for biomechanical modeling purposes. For example, for the arytenoid cartilage, the distance from the muscular process to the apex was measured,[4-6] instead of the distance from the muscular process to the center of the cricoarytenoid joint articular facet, which is biomechanically much more important, as it dictates the moment arm for arytenoid rotational mechanics (in conjunction with the 3-dimensional orientation of the laryngeal abductor and adductor muscles).[10]

The canine larynx has been frequently used as an animal model for the study of the mechanics and physiology of voice production, because of its simi-

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TABLE 1. SUBJECT INFORMATION

	Subject	*Sex*	*Age (y)*	*Weight (kg)*
Humans	H1	M	89	
	H2	M	72	
	H3	M	80	
	H4	M	87	
	H5	M	79	
	H6	M	64	
	H7	M	64	
	H8	M	60	
	H9	M	85	
	H10	F	85	
	H11	F	55	
	H12	F	67	
	H13	F	Unknown	
	H14	F	34	
	H15	F	40	
	H16	F	58	
Dogs	C1	M		24
	C2	F		20
	C3	M		28
	C4	F		25
	C5	M		19
	C6	M		29
	C7	M		23
	C8	M		21
	C9	F		20

larities in size and in basic morphology to the human larynx. For example, data on active contractile properties and passive mechanical properties of the intrinsic laryngeal muscles and the vocal folds have been collected on the canine larynx[11,12] (also Tayama et al, unpublished data), as it is practically very difficult to obtain viable human tissues in vitro (ie, active muscle tissues). Hence, for the purpose of biomechanical modeling, data on geometric features of the canine larynx have been important for comparison with human data. There is a paucity of data on the canine laryngeal framework in the literature,[13] however. This study attempted to augment the work of Eckel et al[5] and Sprinzl et al[6] by defining and reporting new measurements on the human and canine laryngeal frameworks that are deemed critical for developing biomechanical models of the larynx, eg, continuum mechanical models[14] and finite-element models[15] of the vocal folds.

METHOD

TISSUE SPECIMENS

Human Larynges. The excised larynges of 9 men and 7 women were obtained from the autopsy unit of the University of Iowa Hospitals and Clinics (UIHC). They were collected within 24 hours after death, after the stages of rigor mortis in the neck region (Table 1). The ages of the male subjects ranged from 60 to 89 years (mean, 75.6 years), and the ages of the female subjects ranged from 34 to 85 years (mean, 56.5 years). The assumption here was that the gross morphology of the laryngeal framework is not significantly affected by aging (calcification), which is not always true but accurate to the first approximation (see, for example, Malinowski[16]). All larynges were obtained from cadavers with no history of head and neck trauma or diseases of the larynx.

Canine Larynges. Nine canine larynges were obtained after death from UIHC cardiovascular laboratories after other experiments that led to euthanasia of the dogs. The tissues were procured in accordance with the Institutional Animal Care and Use Committee of the University of Iowa. No animals were sacrificed specifically for the present study. The larynges were from large adult mongrel dogs (6 male and 3 female; average weight, 23.2 kg) with no evidence of laryngeal trauma or head and neck diseases (Table 1).

MEASUREMENTS

Geometric measurements on the laryngeal framework that were deemed necessary for modeling purposes were defined and compared with measurements reported in previous studies, including those of Maue and Dickson,[4] Eckel et al,[5] Sprinzl et al,[6] and Eckel and Sittel.[7] The measurements made on each cartilage are illustrated schematically in Figs 1-3.

Thyroid Cartilage. In addition to those measurements defined elsewhere,[4-7] we defined the following biomechanically important measurements on the thyroid cartilage: the left-to-right (L-R) width of the posterior edges at the mid-vertical level (distance between the points 1 and 1' in Fig 1), the anterior-to-posterior (A-P) length (inside) at the vocal fold level, the A-P length (inside) at the inferior (caudal) level (2-2'), the angle between the thyroid laminae (α), the angle between the anterior seam and the posterior cornu (θ), the thickness of the superior thyroid seam (D'-C'), the thickness of the inferior thyroid seam (D-2), and the anterior space (gap) between the thyroid and cricoid cartilages in the midsagittal plane.

Cricoid Cartilage. We defined these additional measurements on the cricoid cartilage: the A-P thickness of the anterior arch (at point G in Fig 2), the medial-lateral (L-R) width of the posterior wall between the centers of the cricothyroid (CT) articular facets (5-5'), the major diameter of the CT articular facet (1-2), the minor diameter of the CT articular facet (3-4), the position of the CT facet in polar coordinates (from point F as origin; rCT, θCT), the po-

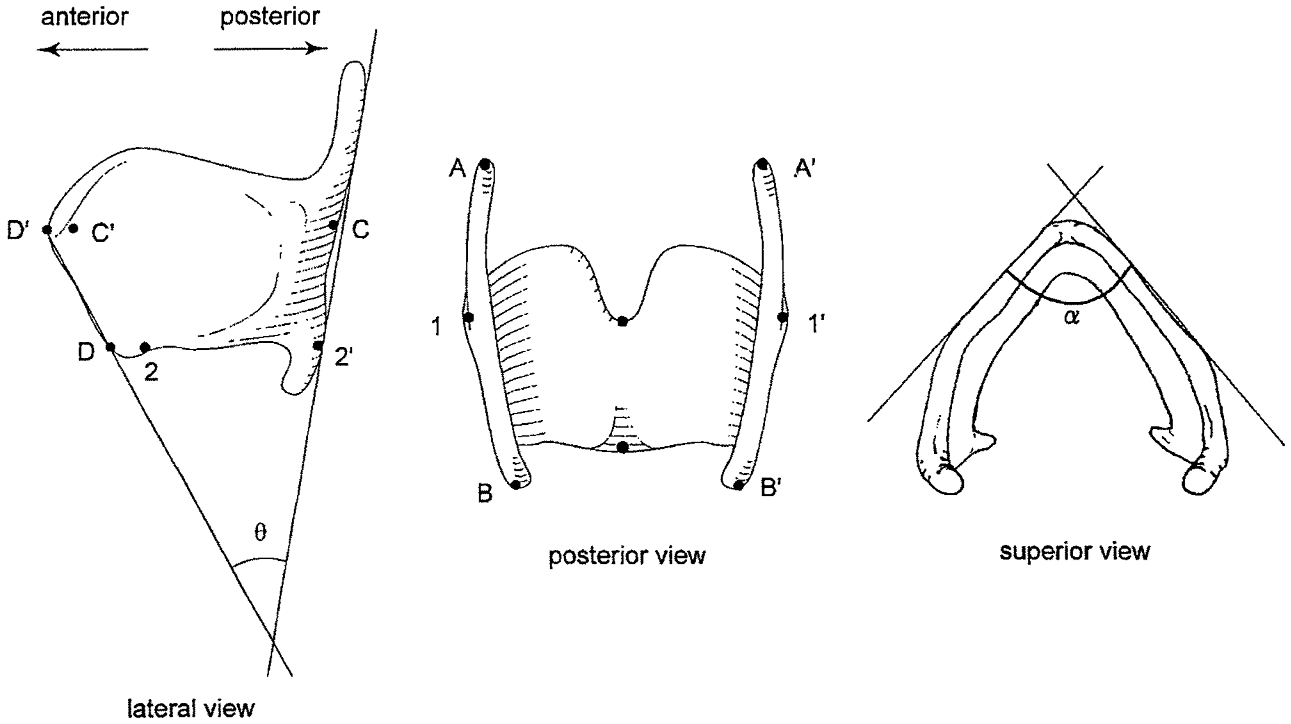

Fig 1. Schematic illustrations of definitions of measurements made on thyroid cartilage.

sition of the CT facet with respect to point F' (r'CT), the position of the cricoarytenoid (CA) articular facet in polar coordinates (from point F as origin; rCA, θCA), and the position of the CA facet with respect to point F' (r'CA). Point F was defined as the most inferior and posterior point of the cricoid cartilage in the midsagittal plane, while point F' was defined as the most superior and posterior point in the midsagittal plane. The geometric positions of the articular facets are important, as they contribute to the determination of the translational and rotational dynamics between the cartilages and, hence, length changes of the vocal folds and pitch control of phonation.[1]

Arytenoid Cartilage. The arytenoid cartilage can be modeled basically in the shape of a prism, with the following additional measurements defined: the distance from the vocal process to the center of the corniculate facet (R-P in Fig 3), the distance from the muscular process to the center of the corniculate facet (S-P), the distance from the vocal process to the center of the CA facet (R-X), the distance from the muscular process to the CA facet center (S-X), and the distance from the apex to the CA facet center (Q-X). The center of the CA facet on the arytenoid cartilage can be assumed to be the axis of rotation or rocking motion for the cartilage on a 2-dimensional plane. Its dimensions and geometric positions with respect to the laryngeal framework are important for determining the rigid body dynamics of the cartilage, eg, for the calculations of moment arms for the posterior CA and lateral CA muscles.[10]

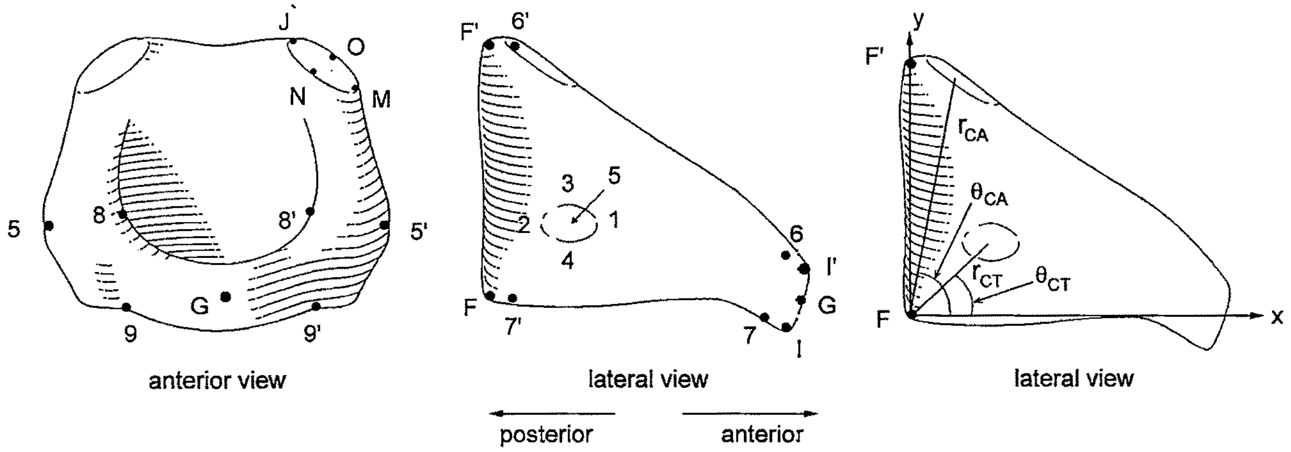

Fig 2. Schematic illustrations of definitions of measurements made on cricoid cartilage.

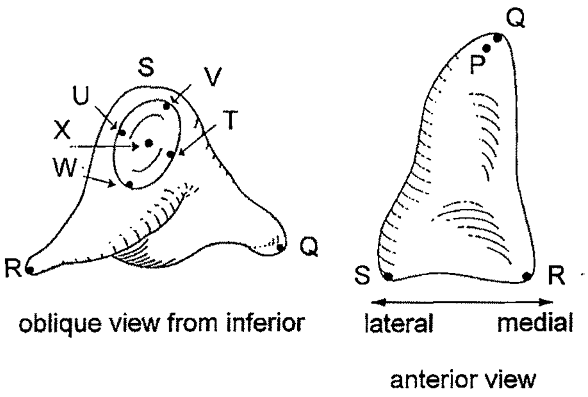

Fig 3. Schematic illustrations of definitions of measurements made on arytenoid cartilage.

First Tracheal Ring. Although this cartilage is not really part of the laryngeal framework, some geometric measurements were made on it, because it is part of the subglottal vocal tract, which is acoustically important for voice production and resonance.[1]

RESULTS AND DISCUSSION

Tables 2, 3, and 4 show the results of all geometric measurements on the thyroid, cricoid, and arytenoid cartilages, respectively. Data are shown as averages and standard deviations. For comparison, data on those measurements that were previously defined and obtained in other studies[4-7] are also shown. As one purpose of the present study was to augment the measurements of Eckel et al[5] and Sprinzl et al,[6] percentage differences between their data and ours were calculated as a means to validate our measurements. In general, there was good agreement between the data sets, with percentage differences often around or below 10%. The only significant discrepancies be-

TABLE 2. THYROID CARTILAGE MEASUREMENTS IN MILLIMETERS

	Men					*Women*					*Dogs*		
*Measurement**	*Mean*	*SD*	*n*	*Reference*	*Diff†* *(%)*	*Mean*	*SD*	*n*	*Reference*	*Diff†* *(%)*	*Mean*	*SD*	*n*
Laryngeal prominence to posterior edges (C-C')	37.27	4.52	6	36.99 ± 2.84[4]	0.76	28.91	NA	2	29.11 ± 6.92[4]	0.69	30.61	1.79	9
Inferior to inferior cornu (B-B')	34.80	4.06	6	35.90 ± 3.90[6]	3.06	25.44	NA	2	29.90 ± 3.60[6]	14.92	27.57	4.20	9
Superior to superior cornu (A-A')	35.50	7.75	5	38.30 ± 7.80[6]	7.30	40.77	NA	2	36.90 ± 6.50[6]	10.47	40.07	4.69	9
Inferior to superior cornu, R (A'-B')	43.07	7.37	5	43.90 ± 4.63[5]	1.89	35.48	NA	2	36.00 ± 3.84[5]	1.44	33.27	3.11	9
Inferior to superior cornu, L (A-B)	46.38	4.58	5	45.10 ± 4.18[5]	2.84	36.10	NA	2	36.50 ± 3.95[5]	1.10	31.80	4.10	9
Left to right width of posterior edges, midvertical (1-1')	41.65	2.82	9			34.05	4.55	7			34.84	4.40	9
Left to right width at vocal fold level (inside)	34.05	2.38	8	32.31 ± 6.22[7]	5.39	28.91	2.89	7	31.56 ± 4.20[7]	8.40	32.44	3.82	8
Anterior to posterior length at vocal fold level (inside)	34.05	3.31	8			27.09	2.07	7			29.73	2.87	9
Anterior to posterior length, bottom (inside; 2-2')	29.70	4.62	9			22.90	2.98	7			25.88	2.78	9
Anterior thyroid notch to inferior border (D-D')	19.24	0.79	6	18.50 ± 2.54[5]	4.00	13.59	NA	2	15.80 ± 1.07[5]	13.99	19.00	2.11	7
Angle between thyroid laminae (α)	77.17	10.15	6	70.25 ± 11.36[7]	9.85	89.80	12.44	5	88.39 ± 9.44[7]	1.60	76.63	7.05	8
Angle between anterior seam and posterior cornu (θ)	35.22	3.46	9			28.60	5.73	5			29.71	6.55	7
Superior thyroid seam thickness (D'-C')	4.13	0.38	6			2.80	0.39	7			2.87	0.57	9
Inferior thyroid seam thickness (D-2)	3.68	1.00	6			2.12	0.47	7			1.96	0.25	9
Anterior gap between thyroid and cricoid cartilages	10.16	2.69	6			8.06	NA	2			11.46	2.18	5

NA — not applicable (standard deviation not computed because of small sample size).

*See Fig 1.

†Percentage differences between our measurements and those from previous studies.

[4] n = 10 for both men and women; from Maue and Dickson.[4]

[5] n = 28 for men and n = 25 for women; from Eckel et al.[5]

[6] n = 28 for men and n = 25 for women; from Sprinzl et al.[6]

[7] n = 12 for men and n = 8 for women; from Eckel and Sittel.[7]

TABLE 3. CRICOID CARTILAGE MEASUREMENTS IN MILLIMETERS

	Men					Women					Dogs		
*Measurement**	*Mean*	*SD*	*n*	*Reference*	*Diff†* *(%)*	*Mean*	*SD*	*n*	*Reference*	*Diff†* *(%)*	*Mean*	*SD*	*n*
Anterior arch, superior-inferior height (I-I')	7.05	1.02	6	6.90 ± 1.35[5]	2.17	5.40	0.01	3	6.20 ± 1.11[5]	12.90	5.31	1.11	9
Anterior arch, anterior-posterior thickness (at G)	3.16	0.72	9			2.58	0.63	7			2.63	0.25	9
Posterior wall, superior-inferior height (F-F')	22.69	1.68	6	24.60 ± 1.90[6]	7.76	19.63	1.65	3	21.60 ± 1.50[6]	9.12	22.52	1.68	9
Posterior wall, left to right width (at CT facets; 5-5')	29.86	2.59	9	29.00 ± 3.20[6]	2.97	21.76	1.82	7	24.40 ± 2.50[6]	10.81	21.10	2.48	9
Inside diameter, anterior-posterior (cranial edge; 6-6')	29.36	2.87	6	21.60 ± 3.61[5]	35.93	23.66	0.23	3	17.40 ± 1.92[5]	35.98	29.40	2.77	9
Inside diameter, anterior-posterior (caudal edge; 7-7')	20.44	2.67	6	20.10 ± 2.30[5]	1.69	17.47	1.20	3	16.40 ± 1.90[5]	6.52	23.44	1.80	9
Inside diameter, left to right (cranial edge; 8-8')	18.26	1.23	6	15.30 ± 1.88[5]	19.35	12.79	0.31	3	12.00 ± 1.23[5]	6.58	15.66	1.97	9
Inside diameter, left to right (caudal edge; 9-9')	22.38	1.43	6	18.20 ± 2.18[5]	22.97	16.82	1.06	3	14.50 ± 1.63[5]	16.00	18.14	2.15	9
Cricothyroid articular facet, major diameter (1-2)	6.15	1.12	9			5.96	0.79	7			5.54	0.91	9
Cricothyroid articular facet, minor diameter (3-4)	4.73	0.72	9			3.73	0.49	7			3.55	0.64	9
Cricoarytenoid articular facet, major diameter (J-M)	8.04	0.74	9	7.72 ± 0.97[4]	4.15	6.85	0.63	7	5.99 ± 0.78[4]	14.36	8.93	1.18	9
Cricoarytenoid articular facet, minor diameter (O-N)	4.07	0.36	9	4.48 ± 0.44[4]	9.15	3.48	0.26	7	3.73 ± 0.48[4]	6.70	3.90	0.53	9
Position of cricothyroid facet in polar coordinates rCT	17.30	1.65	9			14.20	1.60	7			15.52	2.01	9
(F as origin) θCT	45.80	5.59	5			71.33	16.07	3			25.00	2.97	6
(F' as origin) r'CT	22.90	1.78	6			18.41	1.37	6			21.28	1.74	7
Position of cricoarytenoid facet in polar coordinates rCA	23.11	1.06	9			19.84	3.83	7			22.14	2.64	9
(F as origin) θCA	71.17	3.65	6			78.33	2.89	3			65.17	2.34	6
(F' as origin) r'CA	11.37	0.86	6			9.13	0.51	6			9.87	1.43	7

*See Fig 2.

†Percentage differences between our measurements and those from previous studies.

[4]n = 10 for both men and women; from Maue and Dickson.[4]

[5]n = 28 for men and n = 25 for women; from Eckel et al.[5]

[6]n = 28 for men and n = 25 for women; from Sprinzl et al.[6]

tween our data and those of Eckel et al[5] and Sprinzl et al[6] were found in the inside diameters of the cricoid cartilage, for which the differences were as large as 36% (Table 3). Such discrepancies may have arisen from possible between-study differences in the definitions of those measurements.

THYROID CARTILAGE

There was approximately a 12° gender-related difference in the average angle between the thyroid laminae (Table 2), with the larynges of men showing a more acute (smaller) angle (77.17°) than those of women (89.80°). This finding of sexual dimorphism in the human larynx was consistent with those reported previously (eg, a 10° to 20° difference observed by Sprinzl et al[6]). The average thyroid angle in dogs (76.63°) was closer to that of men than to that of women. However, unlike the human larynges, the canine larynges did not have a thyroid notch, regardless of gender. From our informal observations, it seemed that there was not a clear-cut gender differentiation in the dogs. There was also little bilateral asymmetry observed in the dogs.

The A-P length was measured at 3 different levels: the inferior (caudal) level, the vocal fold level, and the level of the thyroid prominence. At the inferior level, it was 25.88 mm for dogs, 29.70 mm for men, and 22.90 mm for women. At the level of the vocal folds, it was 29.73 mm for dogs, 34.05 mm for men, and 27.09 mm for women. At the level of the thyroid prominence, it was 30.61 mm for dogs, 37.27 mm for men, and 28.21 mm for women. At all levels, the dimensions of the canine thyroid cartilage were between those of men and women. The ratio of the A-P lengths at every level can be calculated with

TABLE 4. ARYTENOID CARTILAGE MEASUREMENTS IN MILLIMETERS

	Men					*Women*							
					Diff†					*Diff†*	*Dogs*		
*Measurement**	*Mean*	*SD*	*n*	*Reference*	*(%)*	*Mean*	*SD*	*n*	*Reference*	*(%)*	*Mean*	*SD*	*n*
Vocal process to muscular process (R-S)	15.53	1.23	6	14.33 ± 1.27[4]	8.37	11.05	0.23	3	10.52 ± 1.15[4]	5.04	15.45	1.23	9
Vocal process to corniculate facet center (R-P)	13.91	1.49	8			11.57	1.64	7			11.46	1.27	9
Muscular process to corniculate facet center (S-P)	15.93	1.00	8			13.21	1.29	5			14.27	3.07	9
Vocal process to apex (R-Q)	17.51	2.08	6	17.00 ± 3.67[5]	3.00	12.65	1.24	3	12.70 ± 1.78[5]	0.39	14.56	3.15	7
Muscular process to apex (S-Q)	18.03	1.12	6	17.10 ± 2.44[5]	5.44	14.05	1.51	3	14.00 ± 2.24[5]	0.36	15.65	2.45	8
Cricoarytenoid facet, major diameter (W-V)	6.05	0.44	6	5.68 ± 0.62[4]	6.51	5.47	0.62	3	5.00 ± 0.76[4]	9.40	6.57	1.45	9
Cricoarytenoid facet, minor diameter (T-U)	5.87	0.47	6	5.75 ± 0.44[4]	2.09	3.81	0.36	3	4.52 ± 0.44[4]	15.71	5.22	1.30	9
Vocal process to cricoarytenoid facet center (R-X)	12.36	1.36	8			8.63	1.36	7			10.34	1.41	9
Muscular process to crico-arytenoid facet center (S-X)	5.18	1.13	8			4.49	0.37	4			5.49	0.97	6
Apex to cricoarytenoid facet center (Q-X)	14.84	1.40	8			12.91	1.72	7			13.78	2.35	9

*See Fig 3.

†Percentage differences between our measurements and those from previous studies.

[4] n = 10 for both men and women; from Maue and Dickson.[4]

[5] n = 28 for men and n = 25 for women; from Eckel et al.[5]

respect to the inferior level. The ratios (inferior level to vocal fold level to thyroid prominence level) were 1 to 1.15 to 1.18 for dogs, 1 to 1.15 to 1.25 for men, and 1 to 1.18 to 1.26 for women.

The L-R width was measured at 2 levels: at the vocal fold level and at the level of the thyroid prominence. At the vocal fold level, it was 32.44 mm for dogs, 34.05 mm for men, and 28.91 mm for women. At the level of the thyroid prominence, it was 34.85 mm for dogs, 41.60 mm for men, and 34.05 mm for women. The ratios of the L-R widths (vocal fold level to thyroid prominence level) were 1 to 1.07 for dogs, 1 to 1.22 for men, and 1 to 1.26 for women.

The anterior space (gap) between the thyroid cartilage and the cricoid cartilage was 11.46 mm for dogs, 10.16 mm for men, and 8.60 mm for women. This distance is important for the biomechanical modeling of vocal fold length changes and fundamental frequency (F0) control. It limits the possible amount of CT rotation and translation for increasing the vocal fold length and F0. Our data showed that it seemed to be large enough for substantial relative movements of the cartilages (up to around 10 mm), and hence the possibilities for significant length changes of the vocal folds and a wide range of vocal F0, especially in the human larynx.[1]

CRICOID CARTILAGE

The shape of the canine cricoid cartilage (Table 3) was more flat in the L-R direction, as compared to the ring-like (circular) shape of the human cricoid cartilage. The human cricoid cartilage was shorter in the vertical direction than the canine cricoid cartilage. The height-to-width ratio at the posterior lamina was 1.07 to 1 for dogs, 0.76 to 1 for men, and 0.90 to 1 for women.

The A-P inside diameter of the cricoid at the caudal end was 23.44 mm for dogs, 20.44 mm for men, and 17.47 mm for women. Because the corresponding A-P measurement at the cranial end was oblique, we calculated the approximate values: 23.83 mm for dogs, 24.85 mm for men, and 18.90 mm for women. The ratios of the A-P inside diameters (caudal to cranial) were 1 to 1.01 for dogs, 1 to 1.22 for men, and 1 to 1.08 for women. The L-R inside diameter at the caudal end was 18.14 mm for dogs, 22.38 mm for men, and 16.82 mm for women. The diameter at the cranial end was 15.56 mm for dogs, 18.26 mm for men, and 12.79 mm for women. The ratios of the diameters (caudal to cranial) were 1 to 0.86 for dogs, 1 to 0.82 for men, and 1 to 0.76 for women. The ratios of the L-R diameters to the A-P diameters at the caudal end were 0.77 to 1 for dogs, 1.09 to 1 for men, and 0.96 to 1 for women. The ratios at the cranial end were 0.65 to 1 for dogs, 0.73 to 1 for men, and 0.67 to 1 for women.

As the CT and CA articular facets were roughly elliptical in shape (Fig 2), their dimensions were captured by the major and minor diameters. The ratios of the major diameter to the minor diameter of the CT articular facets were 1.56 to 1 for dogs, 1.30 to 1 for men, and 1.60 to 1 for women. The CA articular facets were more elliptical in shape, with the ratios of the major diameter to the minor diameter being 2.29 to 1 for dogs, 1.98 to 1 for men, and 1.97 to 1 for women. The dimensions and the geometric positions of the CT and CA articular facets with respect to the laryngeal framework are biomechanically important, as they contribute to the determination of CT and CA translational and rotational dynamics, which are critical for length changes and posturing (abduction and adduction) of the vocal folds.

ARYTENOID CARTILAGE

According to most of the measurements in Table 4, the dimensions of the arytenoid cartilage in dogs seemed to be between those in men and women. The CA articular facet on the arytenoid cartilage was much less elliptical (more circular) than its counterpart on the cricoid cartilage, with the ratios of the major diameter to the minor diameter being 1.26 to 1 for dogs, 1.03 to 1 for men, and 1.44 to 1 for women. These data supported the geometric possibility of a sliding motion of the arytenoid on the CA joint along the longitudinal axis of the CA facet surface. For 2-dimensional biomechanical models, if we assume the center of the CA facet to be the axis of rotation (or rocking motion), the distances from the CA facet center to the muscular process and to the vocal process would be very important, as they dictate the moment arms of the laryngeal abductor and adductor muscles for arytenoid rotation, as well as the exact trajectories of the vocal process during vocal fold abduction and adduction.[10]

FIRST TRACHEAL RING

In the dogs, the first tracheal ring was wide, and its posterior part was partly inside the cricoid cartilage, such that this cartilage basically became part of the laryngeal framework. On the other hand, it was smaller in the human subjects, especially in the posterior part. Fusions of the second tracheal ring to the first tracheal ring were also frequently observed in both species.

The A-P diameter (sagittal inner diameter) was 20.16 mm for dogs, 17.63 mm for men, and 14.54 mm for women. The L-R diameter (transverse inner diameter) was 23.95 mm for dogs, 21.55 mm for men, and 16.52 mm for women. It seemed that the first tracheal ring of the dogs was larger than that of the humans, but this finding was inconclusive, because of the frequent fusions between the first tracheal ring and adjacent cartilages. In particular, the A-P diameter was especially affected by fusions, as well as by deformities of the posterior part of the ring. Nonetheless, the dimensions of the first tracheal ring, as well as those of the cricoid cartilage, are helpful for modeling part of the acoustic effects of the subglottal vocal tract on vocal fold vibration.[1]

GENERAL DISCUSSION

Considerable gender-related differences in many of the geometric measurements of the laryngeal framework were observed in the present study, eg, the overall dimensions of the cartilages, the angle between the thyroid laminae, the shape and position of the CT articular facet on the cricoid cartilage, and the shape of the CA articular facet on the arytenoid cartilage. Not only are such gender-related morphological differences important for the purpose of biomechanical modeling, but they also have important clinical implications. For example, they are critical to the accurate placement of laryngeal electromyography electrodes in male versus female patients, as well as the precise planning of laryngeal framework surgery involving thyroid angle alteration and/or precise positioning of the thyroplasty window. However, owing to the presence of a fair amount of intersubject variability in the data (eg, the standard deviation for the thyroid angle in women was 12.44°), these data should be interpreted cautiously, and they may not be universally applicable to individual patients in clinical situations.

CONCLUSION

Geometric data on the laryngeal cartilage framework are important for the biomechanical modeling of the larynx. This study defined and measured some biomechanically important geometric features of the human and canine laryngeal frameworks, augmenting the data of previous studies by Eckel et al[5] and Sprinzl et al.[6] The morphometric data obtained are useful for the construction and refinement of quantitative biomechanical models of vocal fold vibration and posturing (abduction and adduction), eg, continuum mechanical models[14] and finite-element models of the vocal folds.[15]

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CLINICAL NOTE

TREATMENT OF POSTPAROTIDECTOMY SALIVARY FISTULA WITH BOTULINUM TOXIN

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We report on the successful treatment with botulinum toxin type A local injections of a salivary fistula that occurred after superficial parotidectomy. In a 58-year-old woman, transcutaneous discharge of saliva in the preauricular region had persisted in spite of 2 surgical revisions. Moreover, facial weakness and synkinesis had developed as a result of an iatrogenic lesion that had occurred at the time of primary surgery and required immediate reanastomosis of the main nerve trunk. Botulinum toxin A was injected into the deep lobe of the remaining parotid gland under ultrasonographic guidance. Additionally, botulinum toxin A was injected into the left orbicularis oculi muscle in order to improve the synkinesis. No adverse effects were observed. The sialorrhea was stopped for 11 months, and the synkinesis of the facial muscles was reduced significantly for 4 months. We conclude that botulinum toxin A injection is a successful alternative for the treatment of chronic salivary fistula.

KEY WORDS — botulinum toxin, salivary fistula.

INTRODUCTION

The occurrence of salivary fistula is a typical complication following parotid gland surgery. Usually, these fistulas are self-limiting and respond well to pressure dressings applied over several days. However, a chronic transcutaneous discharge of saliva can be observed in rare cases, and there is little consensus on the optimal management of such a condition. In the case reported herein, the injection of botulinum toxin type A into the remaining parotid gland proved to be successful.

CASE REPORT

A 58-year-old woman presented with chronic salivary drainage in the left preauricular region. Superficial parotidectomy for a Warthin's tumor had been performed elsewhere 1 year earlier. Intraoperatively, the main trunk of the facial nerve had been severed, requiring immediate end-to-end nerve anastomosis. Because of persistent sialorrhea in the preauricular region, revision surgery had been performed twice under local anesthesia without success 5 months before referral.

On physical examination, the patient still had a permanent preauricular sialorrhea that increased when she was eating (Fig 1). The left facial nerve showed a moderate dysfunction (House-Brackmann grade III or IV). At rest, the facial symmetry was nearly normal, except for a slight reduction of the palpebral fissure (Fig 2). On motion, there was no movement of the forehead, and the mouth was asymmetric with maximal effort. Electromyography confirmed synkinesis between the orbicular muscle systems of the eye and mouth. On inspection, the postparotidectomy scar was inconspicuous, but Minor's starch-iodine test objectively proved the existence of a preauricu-

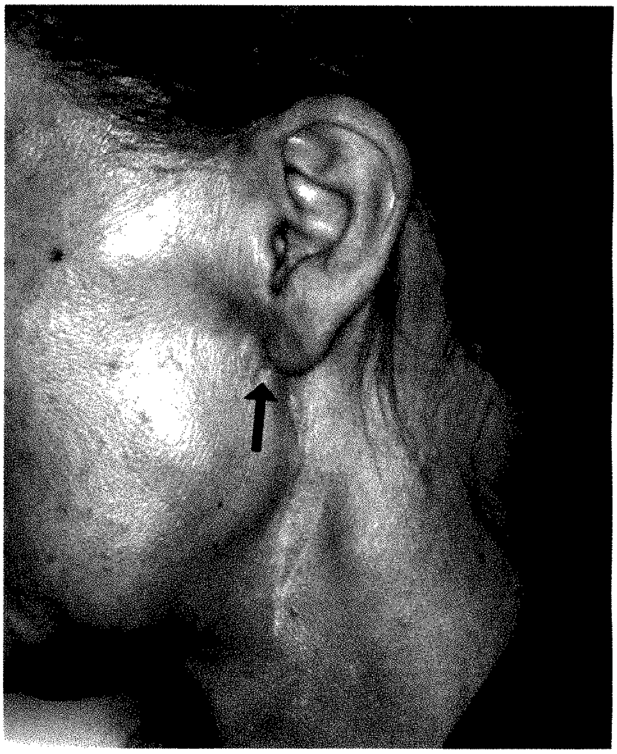

Fig 1. Postparotidectomy scar 1 year after surgery. Note preauricular inflammation due to chronic salivary fistula (arrow).

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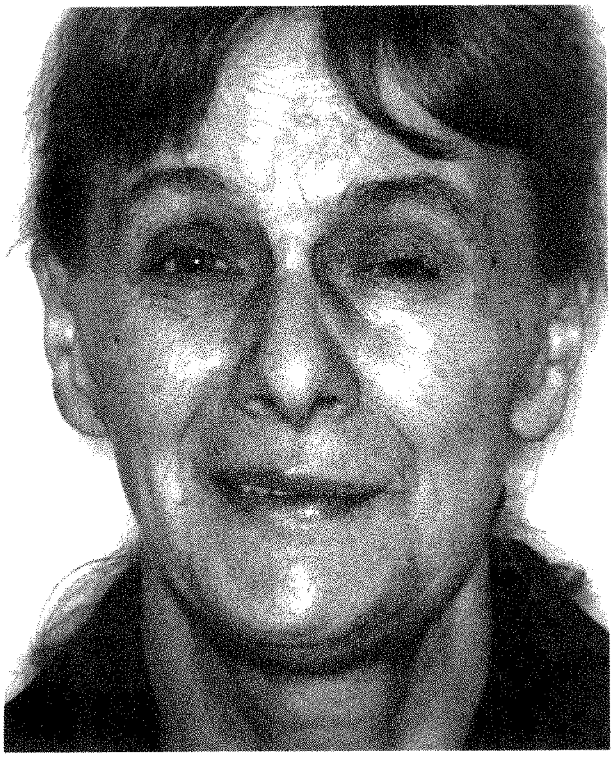

Fig 2. Defective recovery of left facial nerve with synkinesis, perioral weakness, and hyperkinesis of orbicularis oculi muscle 1 year after facial nerve anastomosis because of iatrogenic peripheral nerve lesion.

lar salivary fistula (Fig 3). Magnetic resonance imaging depicted a residual deep lobe of the left parotid gland and a fluid accumulation of 4-mm diameter in the neighboring retroauricular subcutaneous tissue (Fig 4).

Since revision surgery had been performed twice without success and because of the high risk of additional facial nerve damage, the patient refused a third revision operation. Alternatively, the use of botulinum toxin A was suggested to the patient, with the emphasis that there is no formal approval of this substance for the treatment of salivary fistula at this time. After careful discussion, informed consent was obtained for this procedure.

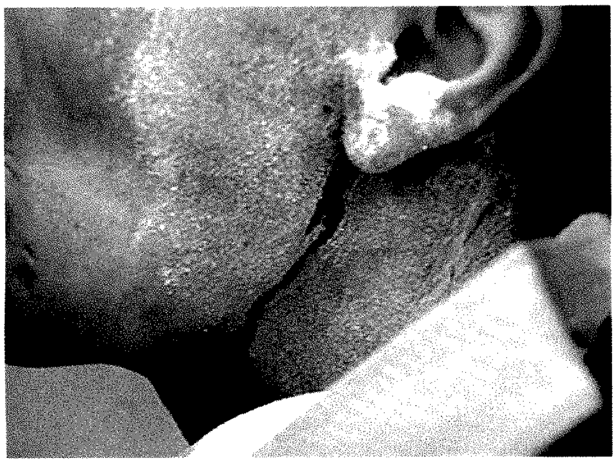

Fig 3. Minor's starch-iodine test objectively shows salivary fistula.

Lyophilized botulinum toxin type A (Dysport) was reconstituted with sterile saline solution to a final concentration of 20 IU/mL. We injected 10 IU of the toxin in the medial and lateral parts of the orbicularis oculi muscles (total dosage, 40 IU). Under ultrasonographic control, we injected 100 IU into the deep lobe of the left parotid gland.

The patient was reexamined monthly over 1 year. No toxin side effects were observed. Subjectively, sialorrhea had ceased after 2 weeks. Minor's starch-iodine test was completely negative 4 weeks after injection. Control electromyography of the orbicularis oculi muscle following botulinum toxin application displayed a decreased firing pattern during voluntary action, especially in the oral region. In the facial musculature, the toxin was effective for 4 months. Hence, application has been repeated in this region approximately every fourth month. Sialorrhea recurred 11 months after the first injection, so parotid gland injection was repeated at the same dosage.

DISCUSSION

Botulinum toxin A has become a relatively safe and effective therapeutic tool in the treatment of a variety of neurologic disorders, especially dystonias.[1] Botulinum toxin prevents the release of acetylcholine at the motor end plates, leading to a reversible partial or complete muscle paralysis. Successful management of cosmetically disturbing synkinesis after defective recovery of facial nerve lesions, as in the case presented, has been added to the list of indications.[2] The major secretomotor fibers to the salivary

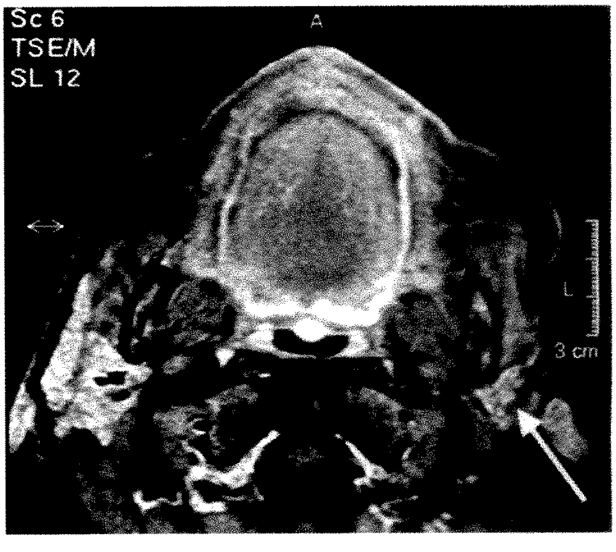

Fig 4. Axial magnetic resonance image depicts residual deep lobe of left parotid gland and adjacent salivary fluid accumulation of 4-mm diameter (large arrow).

glands are parasympathetic and cholinergic. Hence, these fibers are also susceptible to inhibition by botulinum toxin.[3,4] Therefore, injection of this substance is highly effective in the treatment of gustatory sweating.[5,6] The mean duration of this effect is about 17 months. It is believed that botulinum has a longer effect on secretomotor fibers than on pure motor fibers because of differences in the motor end plate physiology and synaptic vesicle turnover, which might explain the long effect of botulinum toxin in the report presented. In the case of treatment for gustatory sweating after parotidectomy, botulinum toxin blocks parasympathetic fibers that have been misdirected to the denervated sweat glands in the surgical field; ie, the toxin blocks the local sweat gland in Frey's syndrome. In contrast, in the presented case, the toxin was used to block the normal, unaffected parasympathetic fibers that had remained in the deep parotid lobe; ie, the toxin stopped the sialorrhea by blocking the residual salivary gland.

Salivary fistula, like gustatory sweating, is a typical complication after partial parotidectomy. However, in most cases the problem is transient, and a chronic salivary fistula is rare condition. Management options include long-term pressure dressings, total parotidectomy, tympanic neurectomy, graft interpositioning, surgical closure of Stensen's duct, radiotherapy, and pharmacotherapy.[7] However, all of these treatment modalities require weeks to months to become effective and impose potential additional risks. In the presented case, revision surgery was not successful. Our observations show that injection of botulinum toxin is a highly effective, relatively safe, and minimally invasive treatment modality with a long-lasting effect. Therefore, selective chemodenervation using botulinum toxin is a successful alternative for the treatment of chronic salivary fistula.

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IMAGING CASE STUDY OF THE MONTH

MANAGEMENT OF A DISPLACED ENDOBRONCHIAL STENT USING SIMULTANEOUS ENDOSCOPY AND TRACHEOBRONCHOGRAPHY

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Airway stents are occasionally placed to manage malacia or stenosis. Advocates of endobronchial stents report efficacy, ease of placement, visibility on plain radiographs, and, for wire mesh stents, preservation of ventilation across bronchial orifices.[1-4] Potential complications include stent fracture or migration, obstructing granulation tissue, perforation of the airway, and erosion into adjacent structures, including vascular structures, that can result in fatal hemorrhage.[1,5-9] Concerns relevant to stent placement in children include effects on bronchial growth and potential complications of long-standing foreign bodies.[10] Attempts at removal may be uncomplicated, but fatal airway obstruction has also been reported.[10]

Tracheobronchography is also a controversial procedure that provides a unified, rather than planar-slice, view of airway anatomy and is unique among radiologic procedures in its capacity to provide dynamic airflow information. Compared to older contrast agents, iohexol, a nonionic contrast agent, is relative-

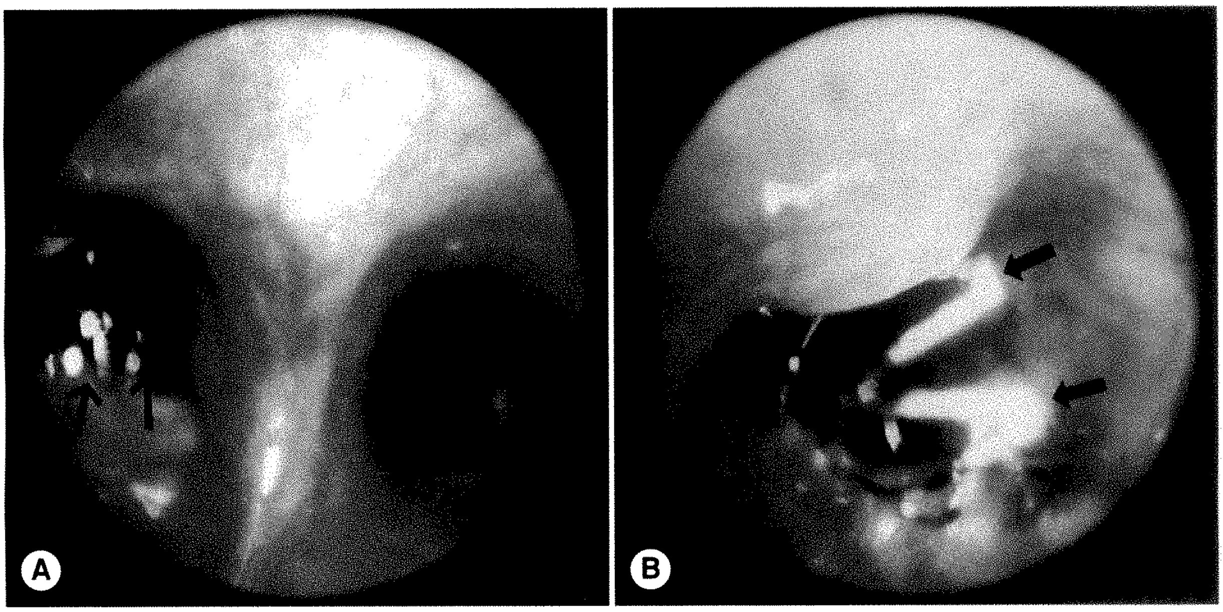

Fig 1. Endobronchial views of proximal end of wire mesh stent. Arrows indicate displaced edges of stent protruding into lumen of left main bronchus **A**) from above carina and **B**) close up. Note minimal mucosal abnormalities.

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Presented as a poster at the meeting of the American Broncho-Esophagological Association, Orlando, Florida, May 15-16, 2000. Awarded the ABEA Presidential Citation for Foreign Body Management.

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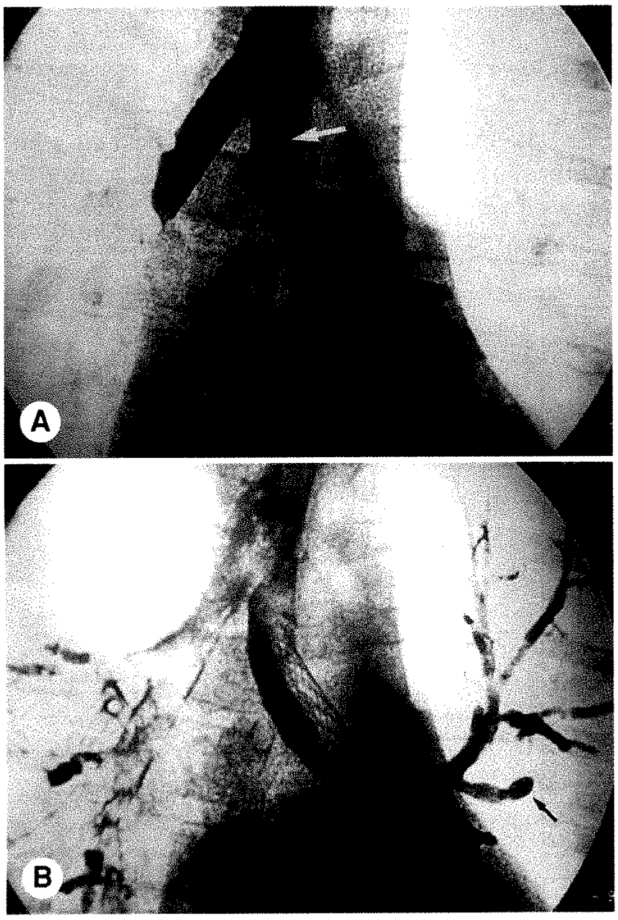

Fig 2. Radiographic images demonstrate flow of tracheobronchography contrast agent (dark material) within trachea and bronchi through wire mesh stent. **A**) Initial image shows good flow beyond displaced portion of stent (arrow). **B**) Subsequent image demonstrates stent details, as well as distal bronchiectasis (arrow).

ly easily administered via a catheter or bronchoscope, provides good contrast, and is well tolerated.[11]

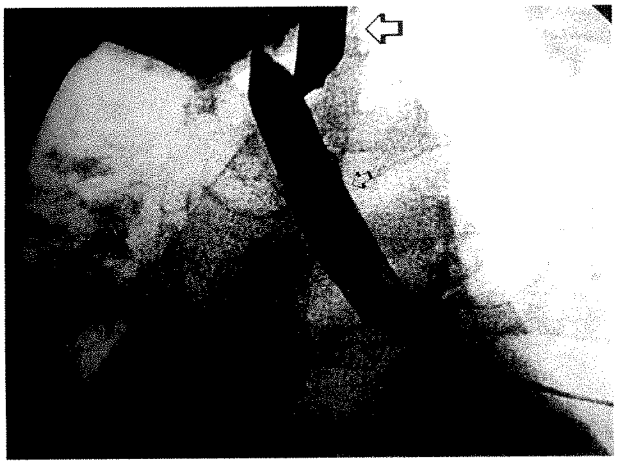

Fig 3. Fluoroscopic view of stent during dilation with expanded vascular balloon catheter (small arrow) and ventilating bronchoscope used for simultaneous endoscopic visualization (large arrow).

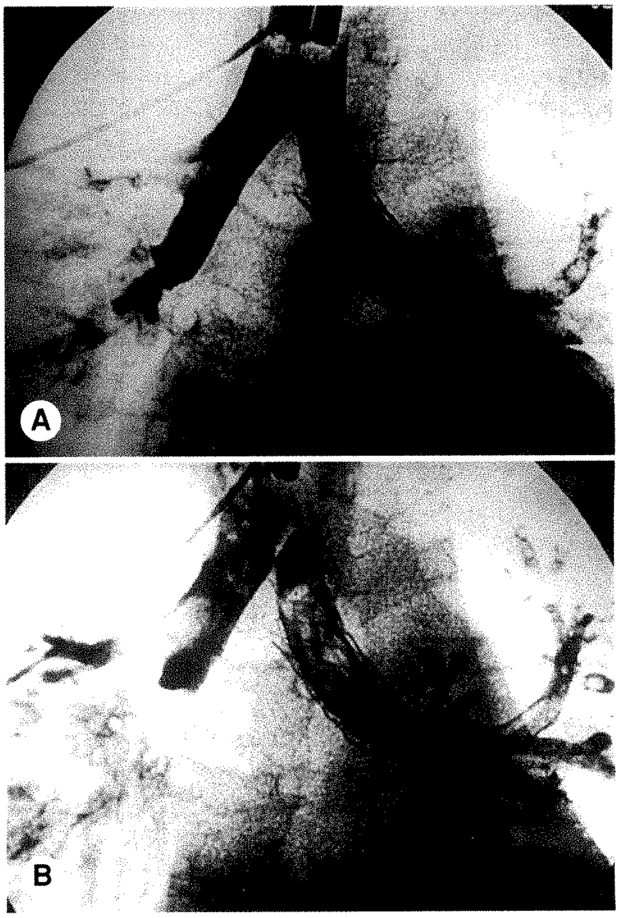

Fig 4. Fluoroscopic images of tracheobronchogram demonstrate improved position of proximal end of stent, with good flow of contrast agent. Distal end of telescope is visible in distal trachea, providing simultaneous endoscopic evaluation. **A**) Initial phase of contrast agent administration. **B**) Later phase of contrast agent administration.

A 5-year-old child with quadriplegia and ventilator dependence following a motor vehicle crash was referred for management of a displaced left main bronchus Palmaz stent, which was originally inserted for management of tracheomalacia and bronchiectasis. Under general anesthesia, rigid bronchoscopy confirmed wire mesh stent malposition, with the proximal end of the stent protruding into the left main bronchus (Fig 1). The mucosa surrounding the stent was normal in appearance. Tracheobronchography, using 6 mL of iohexol (Omnipaque 300, Winthrop Pharmaceuticals, New York, New York) administered via a balloon wedge catheter, revealed adequate airflow through the stent, but severe bronchiectasis (Fig 2). Under simultaneous fluoroscopic and endoscopic guidance, the stent was dilated with a vascular balloon catheter (3 dilations at 10 atmospheres of pressure; Fig 3). The obstructing portion of the stent was expanded and displaced from the lumen of the bron-

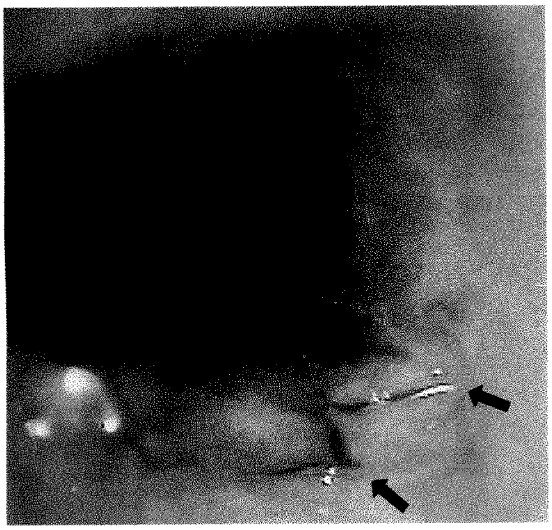

Fig 5. Endoscopic view of proximal end of stent confirms improved position. Arrows indicate good apposition with bronchial wall.

chus, with good airflow confirmed by tracheobronchography using an additional 5 mL of iohexol (Fig 4). Appropriate flaring of the proximal end of the stent and good apposition with the bronchial wall were observed endoscopically (Fig 5). The procedure was well tolerated.

Tracheobronchography provided information about airflow dynamics that could not be obtained endoscopically. Rigid endoscopy provided mucosal and anatomic details that could not be obtained radiographically. Combining the procedures facilitated management of an uncommon airway problem.

We conclude that simultaneous tracheobronchography and rigid endoscopy provided necessary and complementary information that allowed relatively safe and efficacious management of a displaced endobronchial stent.

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MEMORIAL

RAYMOND E. JORDAN, MD

1908-1998

Raymond E. Jordan, MD, died in December 1998 after a long illness. Many of our younger colleagues in otolaryngology will not remember Dr Jordan, because he retired from active participation in otolaryngology in 1972. Nonetheless, Dr Jordan was a very important leader in the development of the field of otology during the 1950s and 1960s. Dr Jordan completed his undergraduate studies at the University of Pittsburgh and graduated from its medical school in 1934. He did postgraduate work in otolaryngology at Washington University in St Louis and was certified by the American Board of Otolaryngology in 1946. Dr Jordan was assigned to the Army Medical Corps and served his country from 1941 through 1945. He was discharged with the rank of Lieutenant Colonel.

When Dr Jordan returned from active duty in 1946, he became a member of the active staff of the Department of Otolaryngology at the Eye and Ear Hospital of Pittsburgh, where he remained until his retirement in 1972. Dr Jordan served as Chairman of the Department of Otolaryngology at the University of Pittsburgh School of Medicine from 1961 to 1972. He served as president of the medical staff of the Eye and Ear Hospital of Pittsburgh from 1959 to 1961. Dr Jordan spent most of his active medical career as an otologist. He studied with Dr Julius Lempert in New York, learning the fenestration operation in the late 1930s. When he came back from the service, he practiced in Pittsburgh with Dr Kenneth Day, Sr, and Dr Kenneth Day, Jr, who also specialized in surgery for deafness. In the middle 1950s, when Dr Samuel Rosen developed the stapes mobilization operation, Dr Jordan was influenced by Dr Rosen and began to practice this form of middle ear surgery for the relief of deafness from otosclerosis. Not long after, Dr John Shea of Memphis introduced the idea of stapedectomy using a vein graft procedure. This technique was the gold standard of middle ear surgery for deafness from otosclerosis for many years, although there were many variations on this theme, and Dr Jordan was one of the pioneer otologists who performed and taught this procedure.

Dr Jordan was very highly recognized for his dedication to teaching and produced many residents from the Eye and Ear Hospital of Pittsburgh who went on to become well-recognized practitioners of otolaryngology.

Dr Jordan held memberships in many societies, including the American Medical Association, the American Academy of Ophthalmology and Otolaryngology, the Pittsburgh Otologic Society, the American Otological Society, the American College of Surgeons, and the Triological Society, of which he was Treasurer from 1963 to 1972. He was also the recipient of many honors and was presented with a Certificate of Award for Distinguished Service in Educational Programs by the American Academy of Ophthalmology and Otolaryngology and was guest of honor of the Triological Society in 1974 and of the Eastern Section of the Triological Society in 1978.

When Dr Jordan retired from his position as Chairman of the Department of Otolaryngology at the University of Pittsburgh School of Medicine in 1972, he moved to Washington, DC, in the role of Executive Director of the American Council of Otolaryngology, which eventually merged with the American Academy of Otolaryngology–Head and Neck Surgery. Because of ill health, he retired from this position in 1974.

Dr Jordan enjoyed a very rich family life and was married for many years to his wife Thelma. They were the parents of two sons and had two grandsons. Years after Thelma passed away, Dr Jordan married the late Jean Dickinson, who had been the wife of John Dickinson, MD, a lifelong professional colleague of Dr Jordan's in Pittsburgh.

Dr Jordan was an excellent surgeon, teacher, and leader in otolaryngology.

EUGENE N. MYERS, MD

Head and Neck Pathology: With Clinical Correlations

Yao-Shi Fu, Bruce Wenig, Elliot Abemayor, and Barry Wenig, editors. Hard cover, illustrated, indexed, 912 pages, 2001. Philadelphia, Pa, Churchill Livingstone, $295.

In the preface to this book, the editors indicate that in the early development of the text, a collaborative effort of both pathologists and clinical specialists was needed to enhance the value beyond that of a book that is primarily pathology-driven. The 4 editors (2 pathologists, 2 surgeons) and their 47 contributing authors have, in the event, produced 839 pages of text, 8 pages of appendices, and 37 pages of index in an attempt to claim the attention of both clinicians and pathologists.

The contents are divided into 2 parts. Part I, entitled "General Principles," presents epidemiology, fine-needle aspiration biopsy, the nowadays-obligatory section on the cellular and molecular biology of the cancer cell, ethical considerations, and a seemingly out-of-place chapter on human immunodeficiency virus and acquired immunodeficiency syndrome.

Part II is entitled "Diseases by Anatomic Site" and entails separate units on the nasal cavity and paranasal sinuses; major salivary glands; larynx, hypopharynx, and trachea; oral cavity and oropharynx; odontogenic lesions; thyroid gland; parathyroid glands; ear and temporal bone; and neck.

Eschewing the trend to multiple volumes, the authors have done all of the above in 1 volume. In their so doing, the book yields to the larger multivolume, interdisciplinary tomes in which a more systematic detailing of pathology and clinical aspects have more room to be expanded and intertwined. The ensuing abbreviation is noted especially in the clinical chapters, eg, the chapter on the nasopharynx, in which the sole emphasis is on management of *recurrent* (emphasis mine) nasopharyngeal cancer, complete with operative technique.

Overlap in discussion points is often not avoidable in texts such as this, but closer editing could have reduced the redundancy in selected areas. As mentioned above, the seemingly out-of-place chapter on HIV and AIDS is too detailed (15 pages), especially since the editors have elected not to explore the hematolymphoid lesions, except for some extranodal ones. The latter subject is well handled, except for the perpetuation of "idiopathic midline destructive disease" as a clinicopathologic entity, a dangerous diagnosis that should be expunged from both clinical and pathology texts.

The mixture of color and black-and-white photographs is appropriate, and the photomicrographs are of fairly even quality.

This text joins the relative abundance of head and neck pathology books that exist either in new editions or as first offerings. It differs somewhat in its attempt to have clinical input. And that raises the question, to whom is this book directed? This reviewer believes it will fare better in the pathology competition.

JOHN G. BATSAKIS, MD
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Controversies in Otolaryngology

Myles L. Pensak, editor. Hard cover, indexed, 476 pages, 2001. Thieme, New York, NY, $129.

Purpose: To show how a group of three experts handle a problem situation. For instance, how would you, the reader, handle an N0 neck? Would you do an elective neck dissection, or would the choice be clinical observation? Reservation of neck dissection until the patient develops regional metastasis? How is otosclerosis managed, and how is Meniere's disease managed?

Contents: Those mentioned in the purpose section are three of the topics. Others include inverting papilloma, chemotherapy, head and neck cancer, uvulopalatopharyngoplasty, free flaps, sinus surgery, quality-of-life issues in cancer, management of the paralyzed face, deep-plane rhytidectomy, skin resurfacing, implantation for nasal reconstruction, ventilation tubes, cholesteatoma, facial paralysis, intracanalicular acoustic neuroma, temporal bone malignancies, perilymphatic fistula, fistula in the head and neck, unknown primary neck disease, carotid neoplasm, pediatric rhinosinusitis, unilateral atretic ear, airway management in the retrognathic patient, otitis media, cochlear implants in the congenitally deaf, and jugular foramen tumors.

Attributes and Limitations: This is a unique form for a book, in that its scope and merit focus to a discussion and a conclusion. There is an absence of illustrations, and a limited bibliography. It is a replica of the 1983 text by a similar name edited by Dr James Snow. The subjects may differ in that, indeed, some have been "laid to rest."

Choosing controversial areas of clinical practice, and offering the opinions of three accomplished practitioners on the same subject, the book should enable the reader to shape a management program of his or her own. In almost all chapters, with the exception of three, the investigators used rational scrutiny to make value judgments. Typically, there is variance in discussion of the disease entity (that is, the three discussions dealing with the same disease process), some being comprehensive, others concise. But this does not detract from the quality of the book. In fact, I think it enhances it, in that one gets a variance in the subject matter among the three authorities.

Are there any ninth-inning collapses? In some chapters, the management scheme seems to stop before it is finished.

Applicability: The volume is designed as both a preview and/or a manual for the otolaryngologist. The reviewer remembers the 1983 text as a viable reference source and will likewise consider the present volume.

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Medical-Legal Evaluation of Hearing Loss

Robert A. Dobie. Second edition. Hard cover, illustrated, indexed, 393 pages, 2001. Singular Thompson Learning, San Diego, Calif, $76.95.

Purpose: To offer current knowledge about noise and its effect on hearing and new directions for hearing conservation services. This volume is a second edition. It has been 7 years since the first edition. The author wanted to update and revise certain features related to the acoustic effects of noise such as advances in hearing research, genetic discoveries, and those newer environmental causes of hearing loss considered recreational (motorcycles) and protective (automobile air bags).

Contents: There are 15 chapters, and the topics within each chapter vary. These chapters can be grouped into 1) medical-audiological sections, 2) recreational hearing trauma, 3) occupational hearing trauma, 4) nonoccupational noise-induced hearing loss, and 5) those subjects that have to do with hearing conservation and legal remedies. Every chapter has been revised, and the author claims there are 150 new references cited.

Included is appendix A ("Typical Noise Levels/Exposures") and appendix B ("Hearing Loss Statutes in the United States and Canada"). Most appreciated of all, appendix C is a list of abbreviations of governmental and audiological terminology.

Attributes and Limitations: The experience and knowledge of Dr Dobie regarding this subject are obvious, in that in every chapter there is informative material in almost every paragraph. This may be a problem with the book, in that the reader may find it difficult to utilize the volume as a reference book. It is information within chapter 13 ("Diagnosis and Allocation") that has to be mastered by the reader. This chapter is worth the price of the book, in that it is a valuable source of information allocating the percentages of hearing loss caused by various traumas such as occupational noise and recreational noise. This chapter is the most revised from its early forerunner. There is better clarity as to how to sort out the percentage of hearing loss that would be allocated to acoustic trauma, versus occupational noise-induced hearing loss. It is done with reasonable clarity, using graphs, tables, and 13 patient examples.

I do not know that much of the new hearing research has been included in this edition, and I really do not know if it is necessary to include it. I think the basic information in anatomy, physiology, and hearing testing included in the book is sufficient for supportive information for medical-legal evaluations of hearing loss. In chapter 4, rather than include such much-used hearing tests as the Békésy test, the Doerfler-Stewart test, the Swing and Story test, electro-dermal audiometry, etc, and take pages of description in doing so, maybe these could be included in a historical review at the beginning of the chapter or be completely eliminated.

Being one myself, I wish that Dr Dobie would eliminate such offensive terms as "old man's hearing" and "old person."

Applicability: This excellent, comprehensive text has a new face and a wealth of information about medical-legal evaluation of hearing loss. It continues to demonstrate why it should be a staple for any student's, otolaryngologist's, or legal counselor's library.

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Endoscopic Anatomy of the Middle Ear

Tschabitscher, Manfred, Klug, Clemens. Hard cover, illustrated, indexed, 127 pages, 2000. Springer, New York, NY, $149.

Purpose: To give an understanding of anatomy through use of today's scopes using the concept of "minimal invasiveness."

Contents: This book is to be considered an atlas. It contains black-and-white photographs, labeled, and to the side, identical photographs, in color, of the same anatomy. There are three such groupings per page. The chapters include the transmeatal, transtympanic, transmastoid, transtubal, and two-port approaches. The various rigid scopes used include 0° and 30° scopes with various known outer diameters. Then a 60° and 70° scope is used. A flexible, nonsteerable scope 0.94 mm in outer diameter and 1,070 mm in length is used, as well as a flexible, steerable scope of 0.8-mm outer diameter and 600-mm length.

Attributes and Limitations: The scopes (endoscopes) have been available since at least the 1920s, when a light source of white ray light was created, eliminating infrared light, which produced too much heat, and ultraviolet light, which was too diffuse. For the next 50 years, rhinology and laryngology were to benefit the most from this invention. It was also in the 1920s that otology forged ahead, introducing the operating microscope. All other specialties lagged for decades in the usage of operative microscopes. Otology lagged in respect to the endoscopic aspect of the technology.

No doubt, the anatomy as seen through endoscopic means is different from that of the operating microscope, in that it is seen at an angle; furthermore, one sees a limited field of view through the scope as compared to the microscope.

As an atlas, this volume meets the criteria for an excellent publication. 1) The quality of the illustrations is superb. 2) The presentation is concise, yet comprehensive and complete. 3) It is extremely focused on anatomy. The authors show confidence and present the material about this recent advance in a meaningful manner. Only time will tell if the endoscope in the middle ear is an inevitable and logical choice.

Applicability: To those with otologic interests.

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